

# Chronic Hepatitis B

ANNA S. F. LOK<sup>1</sup> AND BRIAN J. McMAHON<sup>2</sup>

## PREAMBLE

These guidelines have been written to assist physicians and other health care providers in the recognition, diagnosis, and management of patients chronically infected with the hepatitis B virus (HBV). They are intended to suggest preferable approaches to the clinical management of chronic hepatitis B. The recommendations are flexible and are not intended as the only acceptable approach to management and treatment. As the appropriate course of treatment will vary in light of the relevant facts and circumstances surrounding each individual patient with chronic hepatitis B, guidelines are not intended to define the applicable standard of medical care and may be updated periodically as new information becomes available.

These guidelines were developed under the auspices of, and approved by, the Practice Guidelines Committee of the American Association for the Study of Liver Diseases. They should be taken as guidelines and not “standards of care.” Data used to support the recommendations made were obtained by a literature search of peer-reviewed articles concerning the natural history, diagnosis, and treatment of chronic hepatitis B. In addition, the proceedings of a recent National Institutes of Health workshop on the “Management of Hepatitis B” were considered in the development of these guidelines.<sup>1</sup> The strength of each recommendation is categorized based on the quality of evidence in the literature according to the rating system indicated in Table 1.<sup>2</sup>

## INTRODUCTION

An estimated 350 million persons worldwide are chronically infected with HBV.<sup>3</sup> In the United States, there are an estimated 1.25 million hepatitis B carriers, defined as persons positive for hepatitis B surface antigen (HBsAg) for more than 6 months.<sup>4,5</sup> Carriers of HBV are at increased risk of developing cirrhosis, hepatic decompensation, and hepatocellular carcinoma (HCC).<sup>6</sup> Although most carriers will not develop

hepatic complications from chronic hepatitis B, 15% to 40% will develop serious sequelae during their lifetime.<sup>7</sup> Recommendations in these guidelines pertain to (1) evaluation of patients with chronic HBV infection, (2) prevention of HBV infection, (3) role of HCC surveillance, and (4) treatment of chronic hepatitis B.

## HEPATITIS B VIRUS

HBV belongs to the family of hepadnaviruses. The HBV genome is a relaxed circular, partially double stranded DNA of approximately 3,200 base pairs. There are 4 partially overlapping open reading frames encoding the envelope (pre-S/S), core (precore/core), polymerase, and X proteins.<sup>8,9</sup> The pre-S/S open reading frame encodes the large (L), middle (M), and small (S) surface glycoproteins. The precore/core open reading frame is translated into a precore polypeptide, which is modified into a soluble protein, the hepatitis B e antigen (HBeAg) and the nucleocapsid protein, hepatitis B core antigen. Mutations in the core promoter and precore region have been shown to decrease or abolish HBeAg production.<sup>10,11</sup> The polymerase protein functions as a reverse transcriptase as well as a DNA polymerase. The X protein is a potent transactivator and may play a role in hepatocarcinogenesis.

The replication cycle of HBV begins with the attachment of the virion to the hepatocyte. Inside the hepatocyte nucleus, synthesis of the plus strand HBV DNA is completed and the viral genome is converted into a covalently closed circular DNA (cccDNA). The cccDNA is the template for the pre-genomic RNA, which is reverse transcribed into the minus strand HBV DNA. There are two sources of cccDNA: entry of new virus particles into the hepatocyte and translocation of newly synthesized HBV DNA from the hepatocyte cytoplasm. Most antiviral agents that have been examined so far have little or no effect on cccDNA.<sup>12</sup> This accounts for the rapid reappearance of serum HBV DNA after cessation of antiviral therapy.

## EPIDEMIOLOGY OF HEPATITIS B

Although persons chronically infected with HBV live in all parts of the globe, HBV is especially endemic in Asia, the South Pacific Region, sub-Saharan Africa, in certain indigenous population groups residing in the Arctic (Alaska, Greenland, and Northern Canada), Australia, New Zealand, and populations in South America and the Mid East.<sup>7,13,14</sup> HBV infection is also more prevalent in certain groups in developed countries, such as immigrants from endemic areas, men who have sex with men, injecting drug users, and persons with multiple sex partners.<sup>5,15-19</sup> In some parts of the world such as China and sub-Saharan Africa, HCC associated with HBV is one of the leading causes of cancer in men.<sup>6,7</sup> Table 2 displays the prevalence of HBV serologic markers in population groups that should be screened for HBV infection and immunized if seronegative.

Abbreviations: HBV, hepatitis B virus; HBsAg, hepatitis B surface antigen; HCC, hepatocellular carcinoma; HBeAg, hepatitis B e antigen; cccDNA, covalently closed circular DNA; anti-HBe, antibody to hepatitis B e antigen; ALT, alanine aminotransferase; anti-HBs, antibody to hepatitis B surface antigen; PCR, polymerase chain reaction; HCV, hepatitis C virus; HIV, human immunodeficiency virus; HDV, hepatitis D virus; HBIG, hepatitis B immunoglobulin; AFP, alpha fetoprotein; US, ultrasonography; DCP, des- $\gamma$ -carboxy prothrombin; IFN, interferon.

From the <sup>1</sup>Division of Gastroenterology, University of Michigan Medical Center, Ann Arbor, MI; and the <sup>2</sup>Viral Hepatitis Program, Alaska Native Medical Center and Arctic Investigations Program, Centers for Disease Control, Anchorage, AK.

Received September 14, 2001; accepted September 17, 2001

This guideline has been approved by the American Association for the Study of Liver Diseases and represents the position of the Association. It has also received the endorsement of the American Gastroenterological Association and the American College of Gastroenterology.

Address reprint requests to: Anna S. F. Lok, M.D., Division of Gastroenterology, University of Michigan Medical Center, 3912 Taubman Center, Box 0362, Ann Arbor, MI 48109. E-mail: aslok@umich.edu; fax: 734-936-7392.

This is a US government work. There are no restrictions on its use.

0270-9139/01/3406-0022\$0.00/0

doi:10.1053/jhep.2001.29401

TABLE 1. Quality of Evidence on Which Recommendation is Based

Grade	Definition
I	Evidence from multiple well-designed randomized controlled trials each involving a number of participants to be of sufficient statistical power
II	Evidence from at least one large well-designed clinical trial with or without randomization, from cohort or case-control analytic studies, or well designed meta-analysis
III	Evidence based on clinical experience, descriptive studies, or reports of expert committees
IV	Not rated

NOTE. These definitions of "Quality of Evidence" have been modified by the Practice Guidelines Committee of the American Society for the Study of Liver Diseases from Categories developed by the Infectious Disease Society of America's Quality Standards.<sup>2</sup>

HBV is transmitted by perinatal, percutaneous, and sexual exposure, as well as by close person-to-person contact presumably by open cuts and sores, especially among children in hyperendemic areas.<sup>5,15-17</sup> HBV can survive outside the body for prolonged periods, and carriers who are HBeAg positive can shed large quantities of viral particles ( $10^{7-9}$ ) on environmental surfaces through open cuts or sores.<sup>20,21</sup> The risk of developing chronic HBV infection after acute exposure ranges from 90% in newborns of HBeAg-positive mothers to 25% to 30% in infants and children under 5 and less than 10% in adults.<sup>22-26</sup> In addition, immunosuppressed persons are more likely to develop chronic HBV infection after acute infection.<sup>27,28</sup>

**Recommendations for Persons Who Should Be Screened for HBV Infection:** The following groups should be screened for HBV infection: persons born in hyperendemic areas, men who have sex with men, injecting drug users, dialysis patients, HIV-infected individuals, pregnant women, and family members, household members, and sexual contacts of HBV-infected persons. (II)

#### TERMINOLOGY AND NATURAL HISTORY OF CHRONIC HBV INFECTION

The consensus definition and diagnostic criteria for clinical terms relating to HBV infection adopted at the National Institutes of Health (NIH) workshop on Management of Hepatitis B 2000 are summarized in Table 3.<sup>1</sup>

The most commonly used definition of the carrier state is presence of HBsAg in serum for at least 6 months. It is important to recognize that occasionally it may take a few more months for some individuals to clear HBsAg, but HBsAg

TABLE 3. Glossary of Clinical Terms Used in HBV Infection

Definitions
Chronic hepatitis B Chronic necroinflammatory disease of the liver caused by persistent infection with hepatitis B virus. Chronic hepatitis B can be subdivided into HBeAg-positive and HBeAg-negative chronic hepatitis B.
Inactive HBsAg carrier state Persistent HBV infection of the liver without significant, ongoing necroinflammatory disease.
Resolved hepatitis B Previous HBV infection without further virologic, biochemical, or histologic evidence of active virus infection or disease.
Acute exacerbation or flare of hepatitis B Intermittent elevations of aminotransferase activity to more than 10 times the upper limit of normal and more than twice the baseline value.
Reactivation of hepatitis B Reappearance of active necroinflammatory disease of the liver in a person known to have the inactive HBsAg carrier state or resolved hepatitis B.
HBeAg clearance Loss of HBeAg in a person who was previously HBeAg positive.
HBeAg seroconversion Loss of HBeAg and detection of anti-HBe in a person who was previously HBeAg positive and anti-HBe negative, associated with decrease in serum HBV DNA to $<10^5$ copies/mL.
HBeAg reversion Reappearance of HBeAg in a person who was previously HBeAg negative, anti-HBe positive.
Diagnostic criteria
Chronic hepatitis B
1. HBsAg positive $>6$ months
2. Serum HBV DNA $>10^5$ copies/mL
3. Persistent or intermittent elevation in ALT/AST levels
4. Liver biopsy showing chronic hepatitis (necroinflammatory score $\geq 4$ )*
Inactive HBsAg carrier state
1. HBsAg positive $>6$ months
2. HBeAg negative, anti-HBe positive
3. Serum HBV DNA $<10^5$ copies/mL
4. Persistently normal ALT/AST levels
5. Liver biopsy confirms absence of significant hepatitis (necroinflammatory score $<4$ )*
Resolved hepatitis B
1. Previous known history of acute or chronic hepatitis B or the presence of anti-HBc $\pm$ anti-HBs
2. HBsAg negative
3. Undetectable serum HBV DNA†
4. Normal ALT levels

\*Optional.

†Very low levels may be detectable using sensitive PCR assays.

TABLE 2. Prevalence of HBV Serologic Markers in Population Groups Who Should be Screened for HBV Infection

Population	Prevalence of HBV Serologic Markers (%)	
	HBsAg	All Markers
Persons born in endemic areas	13	70-85
Men who have sex with men	6	35-80
Injecting drug users	7	60-80
Dialysis patients	3-10	20-80
HIV-infected patients	8-11	89-90
Pregnant women (USA)	0.4-1.5	
Family/household and sexual contacts	3-6	30-60

should be undetectable 1 year after acute HBV infection.<sup>25</sup> During the initial phase of chronic HBV infection, serum HBV DNA levels are high and HBeAg is present. The majority of carriers eventually lose HBeAg and develop antibody to HBeAg (anti-HBe).<sup>29-33</sup> In most patients who have undergone seroconversion from HBeAg to anti-HBe, levels of HBV DNA decrease below detection by unamplified assays ( $\sim 10^5$  copies/mL), aminotransferase (ALT) levels normalize, and necroinflammation decreases.<sup>29,32</sup> However, in some patients, liver disease persists or relapses after a period of inactivity. Most of these patients have core promoter or precore variants.

Three serologic patterns of chronic HBV infection have been identified. In Asia and the Oceania, where at least 50% of

chronic HBV infection is a result of perinatal transmission, persistence of HBeAg is longer and seroconversion does not occur in most persons until later in adulthood (pattern 1).<sup>34,35</sup> Among individuals with perinatally acquired HBV infection, a large percent of HBeAg-positive patients have high serum HBV DNA but normal ALT levels.<sup>34,35</sup> These patients are considered to be in the "immune tolerant" phase. Many of these patients develop HBeAg-positive chronic hepatitis B with elevated ALT levels described as pattern 2 in later life.<sup>33,36,37</sup> In sub-Saharan Africa, Alaska, and Mediterranean countries, transmission of HBV usually occurs from person to person in childhood, whereas perinatal transmission is less common (pattern 2).<sup>25,38-40</sup> In these populations most children who are HBeAg positive have elevated ALT levels and seroconversion to anti-HBe is common near or shortly after the onset of puberty. The third pattern is usually observed in individuals who acquired HBV infection during adulthood. This pattern is similar to pattern 2 and is most common in developed countries where sexual transmission is the predominant mode of spread (pattern 3).<sup>15,41</sup> Very little longitudinal data is available on the latter patients, but liver disease is generally present in patients with high HBV DNA levels.<sup>30,32,42</sup>

Among adults in Asia and the Oceania with elevated ALT levels and carriers of all ages with childhood or adult-acquired HBV infection, the rate of clearance of HBeAg averages between 8% and 12% per year.<sup>29-33,43</sup> The rate of clearance of HBeAg is much lower in Asian children (most of whom have normal ALT levels)<sup>34,35</sup> and in immunocompromised subjects.<sup>28,44</sup> The largest prospective follow-up study conducted in Alaska of 1,536 carrier children and adults, followed for 12 years, showed that spontaneous HBeAg clearance occurred in 45% of carriers in 5 years and in 80% after 10 years.<sup>43</sup> Similarly, 3- and 5-year HBeAg clearance rates of 50% and 70% were reported in untreated children with elevated ALT levels from Taiwan and Italy.<sup>36,39</sup> Older age and elevated ALT are predictive of HBeAg clearance. HBeAg clearance may follow an exacerbation of hepatitis, manifested by an elevation of ALT levels.<sup>31,33</sup>

The majority of carriers who develop HBeAg seroconversion remain HBeAg negative and anti-HBe positive with normal ALT levels and minimal or no necroinflammation on liver biopsy. This has been referred to as the "inactive carrier state."<sup>29,32,33,39,40,43,45</sup> The course and outcome of the inactive HBsAg carrier state is generally but not invariably benign depending on the duration and severity of the preceding chronic hepatitis. Because fluctuations in ALT and HBV DNA levels are common during the course of chronic HBV infection, serial tests should be performed before patients are determined to be in an inactive carrier state and periodically thereafter. Up to 20% of carriers in the inactive state can have exacerbations of hepatitis, as evidenced by elevations of ALT levels to 5 to 10 times the upper limit of normal, with or without seroreversion to HBeAg.<sup>33,37,46,47</sup> Repeated exacerbations or reactivations can lead to progressive fibrosis.

HBeAg-negative chronic hepatitis B, characterized by HBV DNA levels detectable by nonamplified assays and continued necroinflammation in the liver, has been reported in all parts of the world but is more common in Mediterranean countries and Asia.<sup>48-64</sup> Most patients with HBeAg-negative chronic hepatitis B harbor HBV variants in the precore or core promoter region.<sup>49-56,59,62-67</sup> The most common precore mutation, G<sub>1896</sub>A, creates a premature stop codon in the precore

region thus abolishing production of HBeAg.<sup>67</sup> This variant is commonly found in association with HBV genotype D, which is prevalent in the Mediterranean basin and is rarely detected in association with HBV genotype A, which is prevalent in the United States and North-West Europe.<sup>51,68</sup> The most common core promoter mutations, A<sub>1762</sub>T + G<sub>1764</sub>A, decrease transcription of precore messenger RNA and production of HBeAg.<sup>11</sup> There are also clinical differences between HBeAg-positive and HBeAg-negative chronic hepatitis B.<sup>57</sup> Patients with HBeAg-negative chronic hepatitis B tend to have lower serum HBV DNA levels and are more likely to run a fluctuating course characterized by persistently elevated or fluctuating ALT levels.<sup>57,60,62</sup>

Approximately 0.5% of HBsAg carriers will clear HBsAg yearly; most will develop anti-HBs.<sup>43,69,70</sup> However, low levels of HBV DNA detectable only by polymerase chain reaction (PCR) assays can be found in up to half of these persons after disappearance of HBsAg.<sup>71</sup> The pathogenic significance of very low levels of HBV DNA is unknown.

One population-based study of HBsAg carriers found the incidence of decompensated cirrhosis to be 0.5 per 1,000 years.<sup>43</sup> In carriers referred to clinical centers, the reported incidence of cirrhosis is as high as 2% to 3% per year possibly because of underlying chronic hepatitis.<sup>61,72-74</sup> Prognostic factors for the development of cirrhosis include HBeAg positivity, older age, and elevated ALT levels.<sup>72,73,75</sup> For patients with compensated cirrhosis, the survival is 84% at 5 years and 68% at 10 years.<sup>75,76</sup> In carriers with decompensated cirrhosis, 5-year survival is only 14%.<sup>76,77</sup> In patients with cirrhosis, risk factors for decompensation include presence of HBeAg and failure to respond to interferon.<sup>77,78</sup> Patients with compensated cirrhosis who were HBeAg-negative had significantly better 5-year survival (97%) than those who were HBeAg-positive (72%).<sup>76</sup> Clearance of HBeAg, whether spontaneous or after antiviral therapy, reduces the risk of hepatic decompensation and improves survival.<sup>72,74,76-81</sup>

Risk factors for HCC in patients with chronic HBV infection include male gender, family history of HCC, older age, presence of cirrhosis, and coinfection with hepatitis C virus (HCV).<sup>6,7,43,77,82</sup> It is important to note that, although HCC is more common in persons with cirrhosis, 30% to 50% of HCC associated with HBV occurs in the absence of cirrhosis.<sup>7</sup> Clearance of HBsAg decreases the risk of hepatic decompensation and probably HCC,<sup>69,83</sup> but HCC can occur in long-term carriers who have cleared HBsAg.<sup>43,70,84</sup>

Coinfection with HCV or human immunodeficiency virus (HIV) is commonly seen in injecting drug users.<sup>5</sup> Coinfection with HIV is also seen in men who have sex with men. Persons who are chronically coinfecting with HBV and HCV may have more rapid progression of liver disease<sup>85</sup> and a higher risk of developing HCC than carriers with HBV infection only.<sup>7</sup> Individuals with HBV and HIV coinfection tend to have higher levels of HBV DNA, lower rates of spontaneous HBeAg seroconversion,<sup>28,44</sup> and more severe liver disease.<sup>86</sup>

Hepatitis D virus (HDV) is a satellite virus, which is dependent on HBV for the production of envelope proteins.<sup>87</sup> HBV/HDV coinfection most commonly occurs in the Mediterranean area and parts of South America. The availability of HBV vaccines and public health education on prevention of transmission of HBV infection has led to a significant decline in the prevalence of HDV infection in the past decade.<sup>88,89</sup> HDV infection can occur in two forms. The first form is caused by the

coinfection of HBV and HDV; this usually results in a more severe acute hepatitis with a higher mortality rate than is seen with acute hepatitis B alone,<sup>87,90</sup> but rarely results in chronic infection. A second form is a result of a superinfection of HDV in an HBV carrier. HDV superinfection can manifest as a severe "acute" hepatitis in previously asymptomatic HBV carriers or exacerbations of underlying chronic hepatitis B. Unlike coinfection, HDV superinfection in HBV carriers almost always results in chronic infection with both viruses. Although persons with chronic HBV/HDV infection can exhibit a wide spectrum of liver pathology, a higher proportion develops cirrhosis, hepatic decompensation, and HCC compared with those with chronic HBV infection alone.<sup>91,92</sup>

## EVALUATION AND MANAGEMENT OF PATIENTS WITH CHRONIC HBV INFECTION

### Initial Evaluation

The initial evaluation of patients with chronic HBV infection should include a thorough history and physical examination, with special emphasis on risk factors for coinfection, alcohol use, and family history of HBV infection and liver cancer. Laboratory tests should include assessment of liver disease, markers of HBV replication, and tests for coinfection with HCV, HDV, and HIV in those at risk (Table 4). Vaccination for hepatitis A should be administered as per Centers for Disease Control recommendations to persons with chronic hepatitis B.<sup>93</sup> Prevacination screening for antibody to hepatitis A (total or IgG) should be considered if the prevalence of infection in the population is likely to be greater than 33%.<sup>93</sup>

**Recommendations for Vaccinating Persons With Chronic HBV Infection Against Hepatitis A:** All persons with chronic hepatitis B not immune to hepatitis A should receive 2 doses of hepatitis A vaccine 6 to 18 months apart.

The appropriate HBV DNA assay to use for initial evaluation of patients with chronic HBV infection has not been determined. An arbitrary value of  $>10^5$  copies/mL was chosen as a diagnostic criterion for chronic hepatitis B at a recent NIH conference.<sup>1</sup> However, there are problems with this definition. First, assays for HBV DNA quantification are not well

standardized (Table 5).<sup>94-96</sup> Second, some patients with chronic hepatitis B have fluctuating HBV DNA levels that may at times fall below  $10^5$  copies/mL. Third, the threshold HBV DNA level that is associated with progressive liver disease is unknown. Quantitative amplification assays can detect HBV DNA levels as low as  $10^2$  copies/mL but the results of these assays have to be interpreted with caution because of the uncertain clinical significance of low HBV DNA levels. Based on our current knowledge and definition of chronic hepatitis B, unamplified assays with detection limits of  $10^5$  to  $10^6$  copies/mL are adequate for the initial evaluation of patients with chronic HBV infection.

The purpose of a liver biopsy is to assess the degree of liver damage and to rule out other causes of liver disease. An international panel of experts recommended that the histologic diagnosis of chronic hepatitis should include the etiology, grade of necroinflammatory activity, and stage/extent of fibrosis.<sup>97</sup> Several numerical scoring systems have been established to permit statistical comparisons of necroinflammatory activity and fibrosis.<sup>98-100</sup> Histologic findings may help in predicting prognosis.<sup>101</sup> However, it must be recognized that liver histology can improve significantly in patients who have sustained response to antiviral therapy or spontaneous HBeAg seroconversion. Liver histology also can worsen rapidly in patients who have recurrent exacerbations or reactivations of hepatitis. Liver biopsies can be used for immunohistochemical staining for HBsAg and hepatitis B core antigen.

### Follow-up of Patients not Considered for Treatment

**HBeAg-Positive Patients With High Serum HBV DNA but Normal ALT Levels.** These patients should be monitored at 3- to 6-month intervals (Table 4). In general, liver biopsy is not necessary unless treatment is contemplated. More frequent monitoring should be performed when ALT levels become elevated. Exacerbations in liver disease have been reported in up to 40% of patients prior to spontaneous HBeAg clearance.<sup>31,33,37,47</sup> Patients who remain HBeAg positive with HBV DNA levels greater than  $10^5$  copies/mL after a 3- to 6-month period of elevated ALT levels should be considered for liver biopsy and antiviral treatment.

**Recommendations for Monitoring Patients With Chronic HBV Infection:** (1) HBeAg-positive patients with elevated ALT levels may be observed for 3 to 6 months for spontaneous seroconversion from HBeAg to anti-HBe prior to initiation of treatment (III).

(2) Patients who meet the criteria for chronic hepatitis B (serum HBV DNA  $>10^5$  copies/mL and persistent or intermittent elevation in aminotransferase levels) should be evaluated further with a liver biopsy (III).

(3) Patients in the inactive HBsAg carrier state should be monitored with periodic liver biochemistries as liver disease may become active even after many years of quiescence (III).

## COUNSELING AND PREVENTION OF HEPATITIS B

Patients with chronic HBV infection should be counseled regarding lifestyle modifications and prevention of transmission. There are no specific dietary measures that have been shown to have any effect on the progression of chronic hepatitis B. However, heavy use of alcohol ( $>40$  g/d) has been associated with higher ALT levels<sup>102,103</sup> and development of cirrhosis.<sup>104</sup> In addition, the development of cirrhosis and HCC occurs at a younger age in heavy drinkers with chronic hepatitis B.<sup>105,106</sup>

TABLE 4. Evaluation of Patients With Chronic HBV Infection

Initial evaluation
History and physical examination
Laboratory tests to assess liver disease—complete blood counts with platelets, hepatic panel, and prothrombin time
Tests for HBV replication—HBeAg/anti-HBe, HBV DNA
Tests to rule out other causes of liver disease—anti-HCV, anti-HDV
Tests to screen for HCC—AFP and, in high risk patients, ultrasound
Liver biopsy to grade and stage liver disease—for patients who meet criteria for chronic hepatitis
Suggested follow-up for patients not considered for treatment
HBeAg-positive chronic hepatitis with HBV DNA $> 10^5$ copies/mL and normal ALT
ALT every 3-6 months
If ALT $>1-2 \times$ ULN, recheck ALT every 1-3 months
If ALT $>2 \times$ ULN for 3-6 months and patient is HBeAg positive, HBV DNA $>10^5$ copies/mL, consider liver biopsy and treatment
Consider screening for HCC in relevant population
Inactive HBsAg carrier state
ALT every 6-12 months
If ALT $>1-2 \times$ ULN, check serum HBV DNA level and exclude other causes of liver disease
Consider screening for HCC in relevant population

TABLE 5. Comparison of HBV DNA Quantification Assays

Assay (Manufacturer)	Volume of Sample	Sensitivity*		Linearity Copies/mL	Genotype Independent	Coefficient of Variation
		pg/mL	Copies/mL			
Branched DNA (Bayer)	10 $\mu$ L	2.1	$7 \times 10^5$	$7 \times 10^5$ - $5 \times 10^9$	A,B,C,D,E,F	6-15%
Hybrid capture (Digene)	30 $\mu$ L 1 mL	0.5 0.02	$1.4 \times 10^5$ $5 \times 10^3$	$2 \times 10^5$ - $1 \times 10^9$ $5 \times 10^3$ - $3 \times 10^6$	A,B,C,D	10-15%
Liquid hybridization (Abbott)	100 $\mu$ L	1.6	$4.5 \times 10^5$ [ $8 \times 10^6$ ] <sup>†</sup>	$5 \times 10^5$ - $1 \times 10^{10}$	Detects genotype D better than A	12-22%
PCR-Amplicor (Roche)	50 $\mu$ L	0.001	$4 \times 10^2$	$4 \times 10^2$ - $1 \times 10^7$ Cobas: $-10^5$ Taqman: $-10^{10}$	(A),B,C,D,E	14-44%
Molecular Beacons	10-50 $\mu$ L	—	<50	50 - $1 \times 10^9$	A-F	5-10%

Adapted from Zuezem S.<sup>25</sup>\*1 pg HBV DNA = 283,000 copies ( $\sim 3 \times 10^5$  viral genome equivalents).<sup>†</sup>Corrected limit of detection.

Carriers of HBV should be counseled as to the risk of transmission to others. Counseling should include precautions to prevent sexual transmission, perinatal transmission, and risk of inadvertent transmission via environmental contamination from a blood spill. Household members are at increased risk of HBV infection and therefore should be vaccinated if they test negative for HBV serologic markers.<sup>5</sup> Screening should be performed by testing for HBsAg and anti-HBs. A positive result for antibody to hepatitis B core antibody does not differentiate between recovered and chronic infection. In addition, false-positive test results are not uncommon in persons with isolated antibodies to hepatitis B core antigen.<sup>107,108</sup> Vaccination of sexual partners has been shown to be effective in preventing sexual transmission of HBV.<sup>5</sup> Steady sexual partners should be tested and vaccinated against hepatitis B if found to be seronegative. For casual sex partners or steady partners who have not been tested or have not completed the full immunization series, barrier protection methods should be employed. HBsAg-positive women who are pregnant should be counseled to make sure they inform their providers so hepatitis B immune globulin (HBIG) and hepatitis B vaccine can be administered to their newborn immediately after delivery.<sup>5</sup> In addition, they should be informed that their infants need to complete the recommended vaccination schedule and have follow-up testing for HBV seromarkers at 1 year of age. HBIG and concurrent hepatitis B vaccine have been shown to be 95% efficacious in the prevention of perinatal transmission of HBV.<sup>16,109</sup> Carriers should be advised to cover open cuts and scratches and clean up blood spills with bleach, because HBV can survive on environmental surfaces for at least 1 week.<sup>20</sup> It should be noted that carriers with high HBV DNA levels are more likely to be infectious, as evidenced by transmission from maternal carriers to infants.<sup>110</sup> Occupational transmission of HBV has also been shown to occur in rare instances.<sup>111,112</sup> For HBV carriers who are health care workers, the Centers for Disease Control recommends that those who are HBeAg-positive should not perform invasive procedures without prior counseling and advice from an expert review panel under what circumstances, if any, they should be allowed to perform these procedures.<sup>113</sup> These circumstances would include notifying prospective patients of their HBV status prior to procedures.

**Recommendations for Prevention of Transmission of Hepatitis B From Individuals With Chronic HBV Infection:** (1) Carriers should be counseled regarding prevention of transmission of HBV. (I)

(2) Sexual and household contacts of carriers should be tested for HBV (HBsAg and anti-HBs) and if negative receive hepatitis B vaccination. (II)

(3) Newborns of HBV-infected mothers should receive HBIG and hepatitis B vaccine at delivery and complete the recommended vaccination series. (I)

(4) Persons who remain at risk for HBV infection such as infants of HBsAg-positive mothers, health care workers, and dialysis patients should be tested for response to vaccination. Infants of carrier mothers should be tested 3 to 9 months, and health care workers 1 to 6 months after vaccination, and dialysis patients should be tested annually. (I)

(5) Abstinence or only limited use of alcohol is recommended in hepatitis B carriers. (III)

#### Periodic Screening for HCC

In longitudinal prospective studies, carriers of HBV have clearly been shown to be at increased risk of developing HCC.<sup>6,7,39</sup> HCC may have a long asymptomatic stage lasting 2 years or longer.<sup>114</sup> In the majority of patients, the cancer begins as a single tumor that is often encapsulated. The doubling time of HCC has been estimated to range from 2 to 12 months with a median of 4 months.<sup>115-117</sup> There is considerable evidence that HCC can be detected early when persons with chronic HBV or HCV infection receive periodic screening. Four population-based screening studies using alpha-fetoprotein (AFP) have been published in HBV carriers, three involving periodic screening and one reporting a one-time mass screening.<sup>118-121</sup> Using AFP as a screening method, small HCC, defined as tumors with a diameter of less than 5 cm, were found in 37% to 59% of persons who had HCC. In clinic-based periodic screening studies involving persons with HBV utilizing both AFP and ultrasound (US) small tumors were found in 57% and 83% of persons respectively with HCC.<sup>122,123</sup> Effective treatment modalities for small HCC have resulted in successful ablation of tumor and reports of long-term tumor-free survival.<sup>124-129</sup>

Patients with small HCC detected by AFP screening and surgically resected who have survived for more than 5 to 10 years have been reported in two population-based studies.<sup>118,119</sup> Duration of tumor-free survival of greater than 5 years would mean that lead time bias is unlikely to be a factor. One of these studies utilizing only AFP compared survival in screened patients with nonscreened historical controls from the same population and showed significant improvement in

5- and 10-year survival rates.<sup>119</sup> Other uncontrolled clinic-based studies have reported long-term survivors who had either surgery or percutaneous ethanol injection after detection of small HCC.<sup>126</sup> Although there is strong evidence that long-term survival can occur in some patients with small HCC that are treated surgically, no randomized trials of carriers undergoing periodic screening compared with those not screened have been reported. In addition, it is important to note that a high false-positive rate of AFP in HBV carriers with chronic hepatitis or cirrhosis may result in expensive evaluations such as radiographic procedures and liver biopsy.

Based on the risk factors discussed in the Natural History section, while it would be easy to identify groups of carriers to prioritize for screening (*i.e.*, men >45 years of age, carriers with cirrhosis or a family history of HCC), carriers of any age, even asymptomatic persons with normal ALT levels and minimal or absent liver disease, can develop HCC. The study from Alaska showed a distinct survival advantage for younger patients detected with HCC, most of whom did not have cirrhosis.<sup>119</sup> However, most HCC develops after decades of chronic HBV infection. Thus, the optimal age to initiate periodic screening is not known.

Several prospective screening studies in HBsAg carriers using laboratory and radiographic tests have been performed.<sup>119-123,130-134</sup> Of the laboratory tests that have been used, AFP has been studied most extensively. The sensitivity of AFP testing depends on the cutoff level employed. The normal level of AFP is less than 8 to 12 ng/mL. If a level of 20 ng/mL is used, the sensitivity for small HCC ranges from 50% to 75%. The specificity of AFP is above 90% in studies that include not only individuals with chronic hepatitis or cirrhosis but also carriers in the inactive state. The negative predictive value is greater than 99%.<sup>119, 122</sup> However, the positive predictive value is low, ranging from 9% to 30%. AFP levels that rise in a step-like manner strongly suggest the presence of HCC, and persons with persistent mild elevation of AFP (<200 ng/mL) are at a higher risk of HCC than those with a single increased value.<sup>119</sup> Other markers that have been shown to be elevated in small HCC in cross-sectional studies include des- $\gamma$ -carboxy prothrombin (DCP), serum- $\gamma$ -glutamyl transferase isoenzyme II, and alpha-L-fucosidase.<sup>135-140</sup> Only DCP has been studied in a prospective manner. Several studies have shown that, while DCP can be elevated in small HCC, the sensitivity of DCP is less than AFP.<sup>135-137</sup> However, two recent studies using a more sensitive assay suggest that DCP and AFP are complimentary and result in a higher sensitivity than either test alone.<sup>141,142</sup> DCP assays are not commercially available in the United States and have not been evaluated as a screening tool.

US is the only radiographic test that has been prospectively studied as an imaging tool for HCC surveillance. Extracting data regarding US from clinic-based studies, the sensitivity for small HCC ranged from 68% to 87% and false-positive rate from 28% to 82%.<sup>122,123,133,134,143</sup> Regenerating nodules, seen in patients with cirrhosis, are the most common reason for false-positive results. US is considerably more expensive than AFP, and, in most developed countries, requires a radiologist. In addition, US is operator dependent and sensitivity of US in detecting small HCC varies depending on the skill of the ultrasound technologist and the radiologist. In addition, large body habitus can make visualization of the liver more difficult and detection of small tumors in cirrhotic livers can be a

challenge. However, US is more sensitive for small HCC than AFP. The combination of AFP and US appears to be superior to either alone but only one randomized trial has been reported, and the number of cases detected and the follow-up period (36 months) was too short to determine if any difference in early detection exists.<sup>122</sup> No randomized trials examining the frequency of HCC surveillance in HBV carriers (or persons with other liver conditions at risk for HCC) have been reported. However, when reviewing the results of 6 clinic-based studies utilizing AFP and US, involving 140 to 1,069 patients with cirrhosis due to HBV or HCV, screening every 6 months appears superior to yearly screening in the detection of small HCC.<sup>122,123,130-133</sup> There appeared to be no difference between screening every 3 or 6 months.

Few cost effectiveness studies on surveillance for HCC in patients with chronic HBV infection have been reported. One clinic-based study from Hong Kong, which has a socialized health care system, using AFP and US for all patients, and computerized tomography for those with AFP levels greater than 20 ng/mL, showed that the cost per tumor detected was \$1,667.<sup>144</sup> In this study using AFP for initial screening 61% of HCC were discovered at a resectable stage. In other studies the cost per tumor detected ranged from \$11,800 to \$25,000.<sup>121,145</sup> In the cohort of carriers from the Alaska study,<sup>146</sup> cost per quality of adjusted life year saved ranged from \$10,000 to \$15,000, well below the widely accepted limit of \$50,000 per quality of adjusted life year gained. However, prospective studies on the cost effectiveness and impact of surveillance for HCC on survival need to be conducted before definitive recommendations on HCC surveillance can be made.

In conclusion, the data available on which to support recommendations for HCC surveillance suggest the following: (1) Periodic testing can detect HCC at a resectable stage in greater than 50% of the instances. (2) Some carriers can experience long-term survival after resection of small HCC, and one study comparing screened cases to historical controls showed a significant survival advantage. (3) Screening with AFP alone has been shown to detect HCC early in some carriers from endemic areas where there is a high risk of perinatal or early childhood infection, and one population study in predominantly noncirrhotic carriers demonstrated 10-year tumor free survival in 27%. (4) US, while more costly, appears to be more sensitive than AFP and the combination of US and AFP may be best. (5) The sensitivity of AFP is less than US but the negative predictive value is high, 99% in low-risk carriers, suggesting that AFP could be used as an initial screening test in low-risk individuals without cirrhosis.<sup>120,122,143</sup> (6) While carriers at higher risk can be identified, all carriers could benefit from periodic testing with AFP. The age to initiate screening for low-risk carriers and the frequency of testing is not known. Evidence to date suggests that carriers with low risk of HCC could be screened with AFP and those at high risk with AFP and US. (7) The age at which screening for HCC should begin is unknown. (8) The optimal frequency for surveillance appears to be every 6 months. The exact risk of HCC in non-endemic populations, such as adult-infected white carriers living in developed countries, has not been determined and the role of periodic screening in this population is not known.

**Recommendations for HCC Screening:** (1) HBV carriers with high risks for HCC such as men over 45 years, persons with cirrhosis, and persons with a family history of HCC, should be screened periodically with both AFP and US. (III)

While there are insufficient data to recommend routine screening in low-risk patients with chronic HBV infection, periodic screening for HCC with AFP in carriers from endemic areas should be considered. (III)

#### TREATMENT OF CHRONIC HEPATITIS B

The aims of treatment of chronic hepatitis B are to achieve sustained suppression of HBV replication and remission of liver disease. The end points used to assess treatment response include normalization in serum ALT level, undetectable serum HBV DNA by an unamplified assay, loss of HBeAg with or without detection of anti-HBe, and improvement in liver histology. Inconsistencies in the definition of response, lack of standardization of HBV DNA assays, and heterogeneity in patient populations make it difficult to compare response rates in clinical trials of treatment of chronic hepatitis B. At the recent NIH workshop on Management of Hepatitis B, it was proposed that responses to antiviral therapy of chronic hepatitis B be categorized as biochemical (BR), virologic (VR), or histologic (HR), and as on-therapy or sustained off-therapy (Table 6).<sup>1</sup> Currently, two therapeutic agents have been approved by the FDA for the treatment of chronic hepatitis B.

#### Interferon

Interferons (IFNs) have antiviral, antiproliferative, and immunomodulatory effects. Interferon alfa (IFN- $\alpha$ ) has been shown to be effective in suppressing HBV replication and in inducing remission of liver disease. However, its efficacy is limited to a small percentage of highly selected patients.

##### Efficacy in Various Categories of Patients.

1. HBeAg-positive chronic hepatitis B with the following:

- a. *Persistent or intermittent elevation in ALT.* This pattern is seen in "typical" chronic hepatitis B patients. A meta-analysis of 15 randomized controlled trials involving 837 adult patients found that a significantly higher percentage of IFN- $\alpha$ -treated patients had a virologic response compared with untreated controls (Table 7).<sup>147</sup> High pretreatment ALT and low serum HBV DNA levels are the most important predictors of a response to IFN- $\alpha$  therapy.<sup>148-150</sup>

TABLE 6. Definition of Response to Antiviral Therapy of Chronic Hepatitis B

Category of response	
Biochemical (BR)	Decrease in serum ALT to within the normal range
Virologic (VR)	Decrease in serum HBV DNA to undetectable levels in unamplified assays (<10 <sup>5</sup> copies/mL), and loss of HBeAg in patients who were initially HBeAg positive
Histologic (HR)	Decrease in histology activity index by at least 2 points compared with pretreatment liver biopsy
Complete (CR)	Fulfill criteria of biochemical and virologic response and loss of HBsAg
Time of assessment	
On-therapy	During therapy
Maintained	Persist throughout the course of treatment
End-of-treatment	At the end of a defined course of therapy
Off-therapy	After discontinuation of therapy
Sustained (SR-6)	6 months after discontinuation of therapy
Sustained (SR-12)	12 months after discontinuation of therapy

TABLE 7. Antiviral Response to Interferon and Lamivudine Therapy in Patients With HBeAg-Positive Chronic Hepatitis B

	Interferon		Lamivudine	
	12-24 Weeks	Controls	52 Weeks	Controls
Loss of serum HBV DNA	37%	17%		
Loss of HBeAg	33%	12%	17%-32%	6%-11%
HBeAg seroconversion	Difference of 18%		16%-18%	4%-6%
Loss of HBsAg	7.8%	1.8%	<1%	0
Normalization in ALT	Difference of 23%		41%-72%	7%-24%
Histologic improvement			49%-56%	23%-25%

b. *Normal ALT.* This pattern is usually seen in children or young adults with perinatally acquired HBV infection. Virologic response to IFN- $\alpha$  therapy is observed in less than 10% of these patients.<sup>150-153</sup>

c. *Asian patients.* Trials in Asian patients with HBeAg-positive chronic hepatitis B found that while the response in patients with normal ALT was poor,<sup>153</sup> the response in patients with elevated ALT was similar to that in white patients.<sup>150</sup>

d. *Children.* The efficacy of IFN- $\alpha$  is similar to that in adults. Among children with elevated ALT, HBeAg clearance has been reported in 30% of those who received IFN- $\alpha$  compared with 10% of controls.<sup>154-156</sup> However, less than 10% of children with normal ALT levels, who received IFN- $\alpha$  cleared HBeAg.<sup>151,152</sup> One meta-analysis of 240 children found that IFN- $\alpha$  treatment increased HBV DNA clearance (odds ratio 2.2), HBeAg clearance (odds ratio 2.2), and ALT normalization (odds ratio 2.3) compared with untreated controls.<sup>157</sup> Adverse events were similar to that in adults.

2. HBeAg-negative chronic hepatitis B

HBeAg loss or seroconversion cannot be used as an end point to assess response in these patients. Therefore, response is usually defined as undetectable serum HBV DNA by unamplified assays and normalization of ALT level. Analyses of the results of trials of IFN- $\alpha$  in HBeAg-negative chronic hepatitis B are complicated by the heterogeneity not just of the disease, but also the virus and study designs. Results of four randomized controlled trials involving a total of 86 IFN- $\alpha$ -treated patients and 84 controls showed that the end-of-treatment response ranged from 38% to 90% in treated patients compared with only 0% to 37% of controls. The 12-month sustained response rates varied from 10% to 47% (average 24%) among the treated patients and 0% in the controls.<sup>158-161</sup> Neither pretreatment factors nor IFN- $\alpha$  dose was predictive of response but longer duration of treatment (12 vs. <6 months) was associated with a doubling of the sustained response rates.<sup>1,162,163</sup> A major problem with IFN- $\alpha$  treatment of HBeAg-negative chronic hepatitis B is relapse, approximately half of the responders relapse when therapy is discontinued, and relapses can occur up to 5 years post-therapy.<sup>164</sup> Nevertheless, sustained response can be achieved in 15% to 25% of patients and long-term follow-up showed that 15% to 30% of sustained responders cleared HBsAg.<sup>1,164</sup>

3. Nonresponders to IFN $\alpha$  treatment

Most studies found that retreatment of IFN- $\alpha$  nonre-

sponders with IFN- $\alpha$  alone was associated with a very low rate of response. However, a recent trial reported an HBeAg clearance rate of 33% among patients retreated with IFN- $\alpha$  versus 10% in untreated controls.<sup>165</sup> Unfortunately, this trial included patients who were previously treated with suboptimal doses of IFN- $\alpha$  and may have overestimated the benefits of IFN- $\alpha$  retreatment.

#### 4. HBV DNA-positive clinical cirrhosis

Approximately 20% to 40% of patients with HBeAg-positive chronic hepatitis B develop a flare in their ALT values during IFN- $\alpha$  treatment. The flare is believed to be a reflection of IFN-induced immune-mediated lysis of infected hepatocytes and is considered to be a predictor of response. In patients with cirrhosis, the flare may precipitate hepatic decompensation. Two studies on IFN- $\alpha$  in patients with Child's class B or C cirrhosis reported no benefit. In addition, significant side effects due to bacterial infection and exacerbation of liver disease occurred even with low doses of IFN- $\alpha$  (3 MU every other day).<sup>166,167</sup> However, IFN- $\alpha$  is safe and may be effective in patients with compensated cirrhosis. In clinical trials of patients with HBeAg-positive chronic hepatitis, up to 60% of patients included had histologic cirrhosis, and less than 1% of patients who received standard doses of IFN- $\alpha$  developed hepatic decompensation.<sup>149,150</sup>

**Dose Regimen.** IFN- $\alpha$  is administered as subcutaneous injections. The recommended dose for adults is 5 MU daily or 10 MU thrice weekly and for children 6 MU/m<sup>2</sup> thrice weekly with a maximum of 10 MU. The recommended duration of treatment for patients with HBeAg positive chronic hepatitis B is 16 to 24 weeks. There are very little data on longer courses of treatment in patients with HBeAg-positive chronic hepatitis B.<sup>168-170</sup> One study found that the response was similar in patients who received 12 versus 24 weeks of IFN- $\alpha$ .<sup>169</sup> Another study reported that among patients who have not cleared HBeAg after 16 weeks of IFN- $\alpha$ , those randomized to continue treatment until week 32 had significantly higher rates of HBeAg clearance compared with those who stopped treatment.<sup>170</sup> Current data suggest that patients with HBeAg-negative chronic hepatitis B should be treated for at least 12 months but it is not clear if longer duration of treatment will increase the rate of sustained response.

**Prednisone Priming.** The rationale for administering a tapering course of steroids prior to antiviral therapy (prednisone priming) is that recovery of immune function following steroid withdrawal may be beneficial particularly if this is timed with the initiation of IFN- $\alpha$  therapy. A meta-analysis of 7 randomized trials of IFN- $\alpha$  with or without prednisone priming in 376 patients with HBeAg-positive chronic hepatitis failed to show a significant benefit of steroid pretreatment.<sup>171</sup> However, a subsequent study of 200 European patients reported that patients who received prednisone priming had a significantly higher rate of HBeAg seroconversion.<sup>172</sup> Although a small subset of patients may benefit from prednisone priming, there is a risk of fatal exacerbations in patients with underlying cirrhosis. Therefore, prednisone priming is not recommended as a primary treatment of chronic hepatitis B.

**Adverse Events.** IFN- $\alpha$  therapy is associated with many adverse effects. Of these, flu-like symptoms, fatigue, leucopenia, and depression are the most common. Most patients develop tolerance to the flu-like symptoms after the first week, but

fatigue, anorexia, hair loss, and mood swings including anxiety, irritability, and depression may persist throughout the course of treatment and for a few weeks after discontinuation of therapy. IFN- $\alpha$  may also unmask or exacerbate underlying autoimmune disorders. An analysis of 9 randomized controlled trials with 552 patients showed that 35% of the patients treated with IFN- $\alpha$  required dose reduction and 5% required premature cessation of treatment.<sup>173</sup>

**Durability of Response and Long-Term Outcome of IFN- $\alpha$ -Treated Patients.** IFN- $\alpha$ -induced HBeAg clearance has been reported to be durable in 80% to 90% of patients after a follow-up period of 4 to 8 years.<sup>80,81,174-176</sup> However, HBV DNA remained detectable in the serum from most of these patients when tested by PCR assays. Five studies in Europe and the United States reported that delayed clearance of HBsAg occurred in 12% to 65% of patients within 5 years of HBeAg loss, but delayed HBsAg clearance was not observed in 2 studies on Chinese patients.<sup>78-81,174,175,177</sup> Sustained virologic response is usually accompanied by a decrease in necroinflammation of the liver but residual hepatic injury is frequently present.<sup>178</sup> Several studies reported that the 5-year cumulative rates of HBeAg clearance were similar in treated patients and controls, but IFN- $\alpha$ -treated patients were more likely to have normal ALT levels and to clear HBsAg.<sup>79,179</sup> These findings suggest that the main role of IFN- $\alpha$  may be to reduce the duration of active liver disease by hastening viral clearance. Data on long-term clinical benefits of IFN- $\alpha$  treatment are limited because chronic hepatitis B is an insidious disease, and adverse outcomes such as progression to cirrhosis, hepatic decompensation, or HCC may not be evident until decades later. In addition, patients initially randomized to the control group frequently receive treatment after completion of the trial. There has been only one report comparing the outcome of treated patients and controls. An 8-year follow-up of 101 male patients who participated in a controlled trial of IFN- $\alpha$  therapy in Taiwan found that treated patients had a lower incidence of HCC (1.5% vs. 12%,  $P = .04$ ) and a higher survival rate (98% vs. 57%,  $P = .02$ ).<sup>80</sup> IFN- $\alpha$  has not been shown to decrease the incidence of HCC in European or North American patients probably because of the low rate of HCC in untreated patients.<sup>79,81</sup> Studies comparing the outcome of responders versus nonresponders found that patients who cleared HBeAg had better overall survival and survival free of hepatic decompensation.<sup>78,80,81</sup>

Data on long-term outcome of patients treated for HBeAg-negative chronic hepatitis B are very limited. It has been estimated that up to 20% of long-term responders cleared HBsAg after 5 years of follow-up.<sup>1,164</sup> In addition, long-term responders appear to have reduced risks of HCC and liver-related deaths.<sup>164</sup>

#### Lamivudine (Epivir-HBV, 3TC)

Lamivudine is the (–) enantiomer of 2'-3'-dideoxy-3'-thiacytidine. Incorporation of the active triphosphate (3TC-TP) into growing DNA chains results in premature chain termination thereby inhibiting HBV DNA synthesis.

##### Efficacy in Various Categories of Adult Patients.

1. HBeAg-positive chronic hepatitis B with the following:
  - a. *Persistent or intermittent elevation in ALT.* Three clinical trials involving a total of 731 treatment naïve patients who received lamivudine for 1 year reported that HBeAg seroconversion (defined as the loss of HBeAg, detection of anti-HBe, and loss of serum



HBV DNA based on non-PCR assays) occurred in 16% to 18% of patients compared with 4% to 6% of untreated controls<sup>180-182</sup> (Table 7). Histologic improvement defined as a reduction in necroinflammatory score greater than 2 points was observed in 49% to 56% treated patients and in 23% to 25% of controls. Follow-up reports of the multicenter Asian study showed that HBeAg seroconversion rates increased with the duration of treatment from 17% at 1 year to 27%, 33%, and 47% at 2, 3, and 4 years, respectively.<sup>183-185</sup> Whether the incremental HBeAg seroconversion can be attributed to the additional years of lamivudine treatment is unclear because most of the patients randomized to placebo in the second year were transferred to open-label lamivudine treatment.

Pretreatment ALT has been found to be the most important predictor of response.<sup>186</sup> Pooled data from 406 patients who received lamivudine 100 mg daily for 1 year showed that HBeAg seroconversion occurred in 2%, 9%, 21%, and 47% of patients with pretreatment ALT levels within normal, 1-2 times normal, 2-5 times normal, and more than 5 times normal.<sup>187</sup> The corresponding figures for 196 patients in the placebo group were 0%, 5%, 11%, and 14%, respectively.

- b. *Normal ALT levels.* HBeAg seroconversion rate after 1 year of treatment is less than 10% in patients with pretreatment ALT levels less than 2 times normal.<sup>186,187</sup>
  - c. *Asian patients.* Asians respond similarly to lamivudine as white patients.
  - d. *Children.* Experience with lamivudine in children is limited. One controlled trial involved 286 children, aged 2 to 17 years, with ALT levels greater than 1.3 times normal. The children were randomized in a 2:1 ratio to lamivudine (3 mg/kg/d up to 100 mg/d) or placebo for 52 weeks. A preliminary report of this trial showed that a significantly higher proportion of treated children developed HBeAg seroconversion compared with placebo controls, 23% versus 13%.<sup>187a</sup> As with adults, the HBeAg seroconversion rate was higher among children with pretreatment ALT levels greater than 2 times normal (34% vs. 16%). The adverse event profile of the two groups was similar. These data indicate that lamivudine is safe and effective in children but the benefit must be carefully balanced against the risk of selecting drug resistant mutants. In this trial, lamivudine-resistant HBV mutants were detected in 18% of treated children.
2. *HBeAg-negative chronic hepatitis B*  
Lamivudine has been shown to benefit patients with HBeAg-negative chronic hepatitis B.<sup>188-193</sup> In one study, virologic and biochemical response was achieved in 34 of 54 (63%) patients who received 24 weeks of lamivudine therapy versus 3 of 53 (6%) patients on placebo ( $P < .001$ ). Of the 54 patients who completed 1 year of lamivudine treatment, serum HBV DNA was undetectable by bDNA assay in 65% and by PCR assay in 39% of patients, and histologic improvement was observed in 60% of patients.<sup>188</sup> Other studies have reported similar

1-year response rates of 70%.<sup>189,191,193</sup> However, the vast majority (~90%) of patients relapsed when treatment was stopped.<sup>194</sup>

### 3. Nonresponders to IFN- $\alpha$ treatment

In a multicenter trial on IFN- $\alpha$  nonresponders, 238 patients were randomized to receive lamivudine monotherapy for 52 weeks, lamivudine for 8 weeks followed by a combination of lamivudine and IFN- $\alpha$  for another 16 weeks, or no treatment. Patients who received lamivudine monotherapy had the highest HBeAg seroconversion rate, 18% compared with 12% and 13%, respectively, in the other groups (not significant).<sup>195</sup> These data suggest that patients who failed IFN- $\alpha$  treatment have a similar response to lamivudine as treatment-naïve patients, and retreatment with combination of IFN- $\alpha$  and lamivudine did not confer any added benefit compared with retreatment with lamivudine monotherapy.

### 4. HBsAg-positive clinical cirrhosis

Studies of lamivudine in patients with decompensated cirrhosis showed that lamivudine treatment is well tolerated and results in clinical improvement in many patients,<sup>196-199</sup> but the optimal timing for initiation of treatment and the subset of patients that are most likely to benefit remain to be determined. In one study of 35 patients (10 with Child-Pugh class C and 25 with Child-Pugh class B), improvement in liver disease defined as a decrease in Child-Pugh score of greater than 2 was observed in 22 of 23 patients who received a minimum of 6 months treatment.<sup>197</sup> However, 7 patients had progressive liver disease necessitating liver transplant and an additional 5 died during the first 6 months. A major concern with early treatment is the selection of resistant mutants. In the study mentioned above,<sup>197</sup> 3 patients developed breakthrough infection. Although all 3 remained clinically stable, more data are needed to determine the long-term outcome of cirrhotic patients who develop lamivudine resistance, their risks of recurrent hepatitis B, and the efficacy of HBIG in the prevention of recurrent hepatitis B after liver transplantation.

**Adverse Events.** In general, lamivudine is very well tolerated. Various adverse events including a mild (2- to 3-fold) increase in ALT level have been reported in patients receiving lamivudine, but these events occurred in the same frequency among the controls.<sup>180-182</sup>

**Durability of Response.** There are very limited data on the durability of HBeAg seroconversion after lamivudine is discontinued. Preliminary data from an ongoing observational study of responders in phase II or III lamivudine trials reported that 34 of 42 (81%) patients with HBeAg seroconversion had durable response after a median follow-up of 21 months (range, 0-30 months). ALT levels were normal in 28 (65%) patients. In addition, 9 (21%) patients had HBsAg seroconversion.<sup>200</sup> However, two studies from Asia reported lower rates of durable response, 38% to 73%.<sup>185, 201</sup> In the study from Korea, 34 patients had HBeAg seroconversion after a mean duration of treatment of  $9.3 \pm 3.0$  months.<sup>201</sup> Post-treatment, the cumulative relapse rates at 1 and 2 years were 38% and 49%. Most, 12 of 16, relapses occurred within the first 12 months after cessation of treatment. Multivariate analysis found that duration of additional lamivudine therapy af-

ter HBeAg seroconversion and pretreatment serum HBV DNA levels were independent predictors for post-treatment relapse.

**Lamivudine Resistance.** Selection of lamivudine-resistant mutants is the main concern with lamivudine treatment. The most common mutation affects the YMDD motif of the HBV DNA polymerase (methionine to valine or isoleucine M204V/I, formerly M552V/I).<sup>202,203</sup> This mutation is frequently accompanied by a leucine to methionine substitution in an upstream region (L180M formerly L528M). Lamivudine resistance is usually manifested as breakthrough infection defined as reappearance of HBV DNA in serum using an unamplified assay on two or more occasions after its initial disappearance. However, breakthrough infection also can be a result of noncompliance. Genotypic resistance can be detected in 14% to 32% after 1 year of treatment.<sup>180-182</sup> In the Asian study, genotypic resistance increased from 14% in year 1 to 38%, 49%, and 66% after 2, 3, and 4 years of treatment, respectively.<sup>183-185</sup> The clinical course of patients with lamivudine resistant mutants is variable and the long-term outcome remains to be determined. In some patients, emergence of lamivudine-resistant mutants may be accompanied by acute exacerbations of liver disease and rarely hepatic decompensation.<sup>204-206</sup> However, most patients who continue treatment have lower serum HBV DNA and ALT levels compared with their pretreatment levels. The continued benefit may be related to the suppressive effect of lamivudine on residual wild-type virus and the impaired replication capacity of the mutants.<sup>207,208</sup> In addition, HBeAg seroconversion has been reported in approximately 25% of the patients who continued treatment after the detection of lamivudine-resistant mutants.<sup>183,204</sup>

The rates of lamivudine resistance in patients treated for HBeAg-negative chronic hepatitis B appear to be more variable (0% to 27% at 1 year and 10% to 56% at 2 years).<sup>188-190,192</sup> More studies are needed to determine the rate of lamivudine resistance in patients treated for HBeAg-negative chronic hepatitis B and the clinical outcome of those who develop breakthrough infection.

**Dose Regimen.** The recommended dose for adults with normal renal function (creatinine clearance >50 mL/min) and no HIV coinfection is 100 mg daily orally. The recommended dose for children is 3 mg/kg/d with a maximum dose of 100 mg/d. Dose reduction is necessary for patients with renal insufficiency. Patients with HIV coinfection should be treated with twice daily 150-mg doses in addition to other anti-retroviral therapies.

The end point of treatment for HBeAg-positive patients is HBeAg seroconversion. In general, lamivudine should be administered for 1 year as a shorter duration of therapy is associated with lower rates of HBeAg seroconversion.<sup>180-182,209,210</sup> Treatment may be discontinued in patients who have completed 1 year of treatment and have persistent HBeAg seroconversion (HBeAg loss, anti-HBe detection, and serum HBV DNA undetectable by non-PCR assays on more than one occasion determined 2-3 months apart). Durability of response after cessation of treatment is expected to be 70% to 80%. Whether lamivudine can be discontinued in patients who have completed 1 year of treatment and have sustained HBeAg loss but no detectable anti-HBe remains to be determined. Based on data from the Korean study,<sup>201</sup> it is not advisable to discontinue treatment before 1 year in patients who have early HBeAg seroconversion.

Treatment may be continued in patients who have not achieved HBeAg seroconversion and have no evidence of breakthrough infection as HBeAg seroconversion may occur with continued treatment.<sup>183</sup> However, treatment beyond 1 year has not been adequately investigated and the benefits of continued treatment must be balanced against the risks of resistant mutants.

Treatment may be continued in patients who have breakthrough infection caused by lamivudine-resistant mutants as long as benefit to the patient (based on clinical assessment, ALT, and HBV DNA levels) is maintained. Patients with worsening liver disease caused by lamivudine-resistant mutants should be referred for clinical trials on potential "rescue" therapy with other nucleoside/nucleotide analogues such as adefovir dipivoxil or entecavir.

Acute exacerbations of hepatitis with or without hepatic decompensation may occur after discontinuation of lamivudine therapy. Exacerbations may occur even in patients who have developed HBeAg seroconversion and may occur up to 1 year (median 4 months) after cessation of treatment.<sup>211</sup> Thus, all patients should be closely monitored for at least 1 year after treatment is discontinued. Reinstitution of lamivudine treatment is usually effective in controlling the exacerbations in patients who have not experienced breakthrough infection and may result in subsequent HBeAg seroconversion,<sup>211</sup> but the benefits of retreatment are usually transient in patients with breakthrough infection as resistant mutants are quickly selected when lamivudine is resumed.<sup>192</sup>

The end point of treatment for HBeAg-negative chronic hepatitis B is unknown. Post-treatment relapse can occur even in patients with undetectable serum HBV DNA by PCR assay. Because of the high rate of relapse in patients who responded after 1 year of treatment, longer duration of treatment may be needed. However, the criteria for discontinuation of treatment has not been determined and the clinical significance of lamivudine resistant mutants in patients with core promoter/pre-core HBV variants remain unclear.

#### Other Therapies

**Famciclovir.** Famciclovir is the oral prodrug of penciclovir. Clinical studies showed that famciclovir is well tolerated and effective in suppressing HBV replication but its antiviral effect is less potent than that of lamivudine. A phase III clinical trial of 417 patients with HBeAg-positive chronic hepatitis B found a higher rate of HBeAg seroconversion compared with controls, 9% versus 3%.<sup>212</sup> Resistance to famciclovir including L180M (L528M) mutation has been reported.<sup>213</sup> In view of the low efficacy, need for thrice daily administration, and potential for cross-resistance with lamivudine, it is unlikely that famciclovir will have a major role in the treatment of chronic hepatitis B.

**Adefovir Dipivoxil.** Adefovir dipivoxil is the prodrug of adefovir. Phase I and II clinical trials showed that adefovir decreased serum HBV DNA levels by 2 to 4 log.<sup>214</sup> Adefovir in high doses is associated with nephrotoxicity. Phase III clinical trials are ongoing to determine the safety and efficacy of lower doses of adefovir. *In vitro* and preliminary clinical data showed that adefovir is effective in suppressing the replication of lamivudine-resistant HBV mutants.<sup>215,216</sup>

**Other Antiviral Agents.** Other antiviral agents that have shown promise in clinical trials include emtricitabine (FTC)<sup>217</sup> and entecavir.<sup>218</sup> *In vitro* studies showed that entecavir is effective against lamivudine-resistant HBV mutants<sup>219</sup>

but there are no published data on its *in vivo* efficacy in patients with breakthrough infection due to these mutants.

**Thymosin.** Thymic-derived peptides can stimulate T-cell function. Clinical trials have shown that thymosin is well tolerated but data on efficacy are conflicting.<sup>220-223</sup> Thus, more studies are needed before thymosin can be recommended for treatment of chronic hepatitis B.

### Combination Therapies

Combination therapies may have additive or synergistic antiviral effects and reduce or delay resistance. Combination therapies have been proven to be more effective in the treatment of chronic HCV and HIV infections. The potential disadvantages of combination therapies include added costs, increased toxicities, and drug interactions.

**IFN- $\alpha$  and Lamivudine.** Combination therapy of IFN- $\alpha$  and lamivudine has been evaluated in two studies. In one study, 226 treatment-naïve patients were randomized to receive lamivudine monotherapy for 52 weeks or IFN- $\alpha$  alone for 16 weeks or lamivudine for 8 weeks followed by lamivudine and IFN- $\alpha$  for 16 weeks. At week 52, the rates of HBeAg seroconversion were 18%, 19%, and 29% in the groups that received lamivudine monotherapy, IFN- $\alpha$  monotherapy, and combination therapy, respectively (not significant).<sup>182</sup> These data indicate that a 1-year course of lamivudine has similar antiviral efficacy to a 16-week course of IFN- $\alpha$  in treatment-naïve patients, and the combination of lamivudine and IFN- $\alpha$  does not seem to have any added benefit. Similar results were reported in the other study on IFN- $\alpha$  nonresponders.<sup>195</sup> However problems in the design of these two studies including sample size, shorter duration of lamivudine therapy (24 vs. 52 weeks) in the group that received combination therapy, and timing of the second biopsy (28 weeks post-treatment vs. on-treatment) prevent a definitive conclusion concerning the efficacy of combination therapy of IFN- $\alpha$  and lamivudine. Studies using other regimens are ongoing. Until further data are available, combination therapy of IFN- $\alpha$  and lamivudine is not recommended.

**Lamivudine and Famciclovir.** *In vitro* and *in vivo* studies in woodchucks showed that lamivudine and penciclovir have additive or synergistic antiviral effects. A pilot study found that a short course of combination therapy of lamivudine and famciclovir have added antiviral efficacy.<sup>224</sup> Whether these effects will translate into higher rates of sustained antiviral response or lower rates of resistant mutants remain to be determined.

### Coinfection With HBV and HDV

The primary endpoint of treatment is the suppression of HDV replication, which is usually accompanied by normalization of ALT level and necroinflammatory activity on liver biopsy. In most countries, the only approved treatment of chronic hepatitis D is IFN- $\alpha$ . Data on the efficacy of IFN- $\alpha$  in chronic hepatitis D are limited. One trial on 61 patients comparing IFN- $\alpha$  in doses of 3 to 5 MU/m<sup>2</sup> 3 times a week for 12 months versus placebo found that there was no difference in sustained virologic response between treated patients and controls, and only 1 patient had sustained biochemical response.<sup>225</sup> Another trial on 42 patients found that patients who received high dose (9 MU 3 times a week) IFN- $\alpha$  had higher rates of virologic and biochemical as well as histologic response.<sup>226</sup> Although most patients had virologic relapse,

improvement in liver histology was maintained 10 years post-treatment among the patients who received high-dose IFN- $\alpha$ .<sup>227</sup>

Lamivudine has been evaluated in a small number of patients and found to be ineffective in inhibiting HDV replication.<sup>228</sup>

Based on available data, high-dose IFN- $\alpha$  (9 MU 3 times a week) for 1 year appears to have long-term beneficial effects in patients with chronic hepatitis D. Because of the rarity of hepatitis D, patients with chronic hepatitis D should be referred to specialized centers for treatment.

**Recommendations for the Treatment of Chronic Hepatitis B:** *Who to treat and what treatment to use (Table 8):* Current therapy of chronic hepatitis B has limited long-term efficacy. Thus, careful balance of patient's age, severity of liver disease, likelihood of response, and potential adverse events and complications is needed before treatment is initiated. Except for patients with decompensated cirrhosis, either IFN- $\alpha$  or lamivudine may be used as initial therapy. The advantages of IFN- $\alpha$  include finite duration of treatment and lack of resistant mutants. The disadvantages of IFN- $\alpha$  are the costs and side effects. Lamivudine is more economical (if given for 1 year only) and well tolerated but the durability of response and the long-term clinical significance of the resistant mutants are uncertain.

1. Patients with HBeAg-positive chronic hepatitis B
  - a. ALT greater than 2 times normal or moderate/severe hepatitis on biopsy. These patients should be considered for treatment. Treatment may result in virologic, biochemical, and histologic response (I) and also appear to improve clinical outcome (II). Treatment may be initiated with lamivudine or IFN- $\alpha$  as the 2 treatments have similar efficacy.
  - b. ALT persistently normal or minimally elevated (<2 times normal). These patients should not be initiated on treatment unless there is significant necroinflammation on liver biopsy (II).
  - c. Children with elevated ALT greater than 2 times normal. These patients should be considered for treat-

TABLE 8. Recommendations for Treatment of Chronic Hepatitis B

HBeAg	HBV DNA*	ALT	Treatment Strategy
+	+	<2 $\times$ normal	Low efficacy for both IFN- $\alpha$ and lamivudine treatment. Observe patient, consider treatment when ALT becomes elevated.
+	+	>2 $\times$ normal	IFN- $\alpha$ or lamivudine therapy. In IFN- $\alpha$ nonresponders and patients with contraindications to IFN- $\alpha$ , lamivudine is preferred.
–	+	>2 $\times$ normal	IFN- $\alpha$ or lamivudine. Long-term treatment required.
–	–	<2 $\times$ normal	No treatment required.
+/-	+	Cirrhosis	Compensated: IFN- $\alpha$ (close monitoring required) or lamivudine Decompensated: Lamivudine treatment. Optimal timing of therapy unknown. Liver transplantation.
+/-	–	Cirrhosis	Compensated: Observe. Decompensated: Liver transplantation.

\*HBV DNA >10<sup>5</sup> copies/mL. This value is arbitrarily chosen and may be lower for patients with HBeAg-negative chronic hepatitis B and those with decompensated cirrhosis.

ment (II). Both IFN- $\alpha$  and lamivudine are approved treatments for children with chronic hepatitis B.

2. Patients with HBeAg-negative chronic hepatitis B (serum HBV DNA  $>10^5$  copies/mL, elevated ALT  $>2$  times normal or moderate/severe hepatitis on biopsy) should be considered for treatment (II). Treatment may be initiated with lamivudine or IFN- $\alpha$  (II).
3. Patients who failed to respond to prior IFN- $\alpha$  therapy may be retreated with lamivudine if they fulfill the criteria listed above (II).
4. Patients with decompensated cirrhosis may be considered for lamivudine treatment (III). Treatment should be coordinated with transplant centers. IFN- $\alpha$  should not be used in patients with decompensated cirrhosis (II).
5. In patients with inactive HBsAg carrier state antiviral treatment is not indicated.

#### Dose Regimens.

1. IFN- $\alpha$  is administered as subcutaneous injections.
  - a. *The recommended IFN- $\alpha$  dose for adults is 5 MU daily or 10 MU thrice weekly.*
  - b. *The recommended IFN- $\alpha$  dose for children is 6 MU/m<sup>2</sup> thrice weekly with a maximum of 10 MU.*
  - c. *The recommended treatment duration for HBeAg-positive chronic hepatitis B is 16 weeks.*
  - d. *The recommended treatment duration for HBeAg-negative chronic hepatitis B is 12 months.*
2. Lamivudine is administered orally.
  - a. *The recommended lamivudine dose for adults with normal renal function and no HIV coinfection is 100 mg daily.*
  - b. *The recommended lamivudine dose for children is 3 mg/kg/d with a maximum of 100 mg/d.*
  - c. *The recommended treatment duration for HBeAg-positive chronic hepatitis B is 1 year.* Treatment may be continued in patients who have not developed HBeAg seroconversion but treatment beyond 1 year should balance the benefits against the risks of resistant mutants. Treatment may be continued in patients who have breakthrough infection due to lamivudine-resistant mutants as long as benefit to the patient (based on clinical assessment, ALT, and HBV DNA level) is maintained.
  - d. *The recommended treatment duration for HBeAg negative chronic hepatitis B is longer than 1 year but the optimal duration has not been established.*

#### APPENDIX

The Practice Guidelines Committee Members are as follows: Henry C. Bodenheimer, Jr., MD (Chair); David Eric Bernstein, MD; Gary L. Davis, MD; James Everhart, MD; Stuart C. Gordon, MD; F. Blaine Hollinger, MD; Donald M. Jensen, MD; Maureen Jonas, MD; Jacob Korula, MD; Timothy M. McCashland, MD; Jan M. Novak, MD; Melissa Palmer, MD; Rajender Reddy, MD; Eve A. Roberts, MD; James R. Spivey, MD; Thomas Shaw-Stiffel, MD.

#### REFERENCES

1. Lok AS, Heathcote EJ, Hoofnagle JH. Management of Hepatitis B 2000, Summary of a Workshop. *Gastroenterology* 2001;120:1828-1853.
2. Gross PA, Barrett TL, Dellinger EP, Krause PJ, Martone WJ, McGowan JE Jr., Sweet RL, et al. Infectious Diseases Society of America quality standards for infectious diseases: purpose of quality standards for infectious diseases. *Clin Infect Dis* 1994;18:421.
3. Lee W. Hepatitis B virus infection. *N Engl J Med* 1997;337:1733-1745.
4. McQuillan GM, Townsend TR, Fields HA, Carrol M, Leahy M, Polk BF. Seroepidemiology of hepatitis B virus infection in the United States. *Am J Med* 1989;87(suppl 3A):5S-10S.
5. CDC. Hepatitis B virus: a comprehensive strategy for limiting transmission in the United States through universal childhood vaccination. Recommendations of the Immunization Practices Advisory Committee (ACIP). *MMWR* 1991;40:RR-13:1-25.
6. Beasley RP. Hepatitis B virus: the major etiology of hepatocellular carcinoma. *Cancer* 1988;61:1942-1956.
7. McMahon BJ. Hepatocellular carcinoma and viral hepatitis. In: Wilson RA, ed. *Viral Hepatitis*. New York: Marcel Dekker 1997;315-330.
8. Seeger C, Mason WS. Hepatitis B virus biology. *Microbiol Mol Biol Rev* 2000;64:51-68.
9. Ganem D, Schneider RJ. Hepadnaviridae and their replication. In: Knipe DM, Howley PM, Chanock RM, Monath TP, Roizman B, Straus SE, eds. *Fields Virology*. 4th ed. Philadelphia: Lippincott-Raven, 2001:2703-2737.
10. Scaglioni PP, Melegari M, Wands JR. Biologic properties of hepatitis B viral genomes with mutations in the precore promoter and precore open reading frame. *Virology* 1997;233:374-381.
11. Buckwold VE, Xu Z, Chen M, Yen TS, Ou JH. Effects of a naturally occurring mutation in the hepatitis B virus basal core promoter on precore gene expression and viral replication. *J Virol* 1996;70:5845-5851.
12. Locarnini S, Birch C. Antiviral chemotherapy for chronic hepatitis B infection: lessons learned from treating HIV-infected patients. *J Hepatol* 1999;30:536-550.
13. Maynard JE. Hepatitis B: global importance and need for control. *Vaccine* 1990;8(Suppl):S18-S20.
14. Mast EE, Alter MJ, Margolis HS. Strategies to prevent and control hepatitis B and C virus infections: a global perspective. *Vaccine* 1999;17:1730-1733.
15. Margolis HS, Alter MJ, Hadler SC. Hepatitis B: evolving epidemiology and implications for control. *Semin Liver Dis* 1991;11:84-92.
16. CDC. Recommendations for protection against viral hepatitis. Recommendations of the Immunization Practices Advisory Committee (ACIP). *MMWR* 1985;34:313-335.
17. CDC. Prevention of perinatal transmission of hepatitis B virus: prenatal screening of all pregnant women for hepatitis B surface antigen. Recommendations of the Immunization Practices Advisory Committee (ACIP). *MMWR* 1988;37:341-346.
18. Schar Schmidt BF, Held MJ, Hollander HH, Read AE, Lavine JE, Veerman G, McGuire RF, et al. Hepatitis B in patients with HIV infection: relationship to AIDS and patient survival. *Ann Intern Med* 1992;117:837-838.
19. Rodriguez-Mendez ML, Gonzalez-Quintela A, Aguilera A, Barrio E. Prevalence, patterns and course of past hepatitis B virus infection in intravenous drug users with HIV-1 infection. *Am J Gastroenterol* 2000;95:1316-1322.
20. Bond WW, Favero MS, Petersen NJ, Gravelle CR, Ebert JW, Maynard JE. Survival of hepatitis B virus after drying and storage for one week [letter]. *Lancet* 1981;1:550-551.
21. Petersen NJ, Barrett DH, Bond WW, Berquist KR, Favero MS, Bender TR, Maynard JE. Hepatitis B surface antigen in saliva, impetiginous lesions, and the environment in two remote Alaskan villages. *Applied Environ Microbiol* 1976;32:572-574.
22. Beasley RP, Hwang LY, Lee GCY, Lin CC, Roan CH, Huang FY, Chen CL. Prevention of perinatally transmitted hepatitis B virus infections with hepatitis B immune globulin and hepatitis B vaccine. *Lancet* 1983;1:1099-1102.
23. Beasley RP, Hwang LY, Lin CC, Leu ML, Stevens CE, Szmuness W, Chen KP. Incidence of hepatitis B virus in preschool children in Taiwan. *J Infect Dis* 1982;146:198-204.
24. Corsaget P, Yvonnet B, Chotard J, Vincelot P, Sarr M, Diouf C, Chiron JP, et al. Age- and sex-related study of hepatitis B virus chronic carrier state in infants from an endemic area (Senegal). *J Med Virol* 1987;22:1-5.
25. McMahon BJ, Alward WLM, Hall DB, Heyward WL, Bender TR, Francis DP, Maynard JE. Acute hepatitis B virus infection: Relation of age to the clinical expression of disease and subsequent development of the carrier state. *J Infect Dis* 1985;151:599-603.
26. Tassopoulos NC, Papaevangelou GJ, Sjogren MH, Roumeliotou-Karayannis A, Gerin JL, Purcell RH. Natural history of acute hepatitis B

- surface antigen-positive hepatitis in Greek adults. *Gastroenterology* 1987;92:1844-1850.
27. Horvath J, Raffanti SP. Clinical aspects of the interactions between human immunodeficiency virus and the hepatotropic viruses. *Clin Infect Dis* 1994;18:339-347.
  28. Bodsworth N, Donovan B, Nightingale BN. The effect of concurrent human immunodeficiency virus infection on chronic hepatitis B: a study of 150 homosexual men. *J Infect Dis* 1989;160:577-582.
  29. Hoofnagle JH, Dusheiko GM, Seeff LB, Jones EA, Waggoner JG, Bales ZB. Seroconversion from hepatitis B e antigen to antibody in chronic type B hepatitis. *Ann Intern Med* 1981;94:744-748.
  30. Viola LA, Harrison IG, Coleman JC, Paradinal FJ, Fluker JL, Evans BA, Murray-Lyon IM. Natural history of liver disease in chronic hepatitis B surface antigen carriers: survey of 100 patients from Great Britain. *Lancet* 1981;2:1156-1159.
  31. Liaw YF, Chu CM, Su IJ, Huang MJ, Lin DY, Chang-Chien CS. Clinical and histological events preceding hepatitis B e antigen seroconversion in chronic type B hepatitis. *Gastroenterology* 1983;84:216-219.
  32. Fattovich G, Rugge M, Brollo L, Pontisso P, Noventa F, Guido M, Alberti A, et al. Clinical, virologic and histologic outcome following seroconversion from HBeAg to anti-HBe in chronic hepatitis type B. *HEPATOLOGY* 1986;6:167-172.
  33. Lok ASF, Lai CL, Wu PC, Leung EKY, Lam TS. Spontaneous hepatitis e antigen to antibody seroconversion and reversion in Chinese patients with chronic hepatitis B virus infection. *Gastroenterology* 1987;92:1839-1843.
  34. Lok AS, Lai CL. A longitudinal follow-up of asymptomatic hepatitis B surface antigen-positive Chinese children. *HEPATOLOGY* 1988;8:1130-1133.
  35. Chang MH, Hsu HY, Hsu HC, Ni YH, Chen JS, Chen DS. The significance of spontaneous hepatitis e antigen seroconversion in childhood: with special emphasis on the clearance of hepatitis e antigen before 3 years of age. *HEPATOLOGY* 1995;22:1387-1392.
  36. Lee PI, Chang MH, Lee CY, Hsu HY, Chen JS, Chen PJ, Chen DS. Changes in serum hepatitis B DNA and aminotransferase levels during the course of chronic hepatitis B virus infection in children. *HEPATOLOGY* 1990;12:657-660.
  37. Lok ASK, Lai CL. Acute exacerbations in Chinese patients with chronic hepatitis B (HBV) virus infection: Incidence, predisposing factors and etiology. *J Hepatol* 1990;10:29-34.
  38. Dusheiko GM, Brink BA, Conradie JD, Marimuthu T, Sher R. Regional prevalence of hepatitis B, Delta, and human immunodeficiency virus infection in Southern Africa: a large population survey. *Am J Epidemiol* 1989;129:138-145.
  39. Bortolotti F, Cadrobbi P, Crivellaro C, Guido M, Rugge M, Noventa F, Calzia R, et al. Long-term outcome of chronic type B hepatitis in patients who acquire hepatitis B infection in childhood. *Gastroenterology* 1990;99:805-810.
  40. Moreno MR, Otero M, Millan A, Castillo I, Cabrero M, Jimenez FJ, Oliva H, et al. Clinical and histological outcome after hepatitis B e antigen to antibody seroconversion in children with chronic hepatitis B. *HEPATOLOGY* 1999;29:572-575.
  41. Stroffolini T, Mele A, Tosti ME, Gallo G, Balocchini E, Ragni P, Santonastasi F, et al. The impact of hepatitis B mass immunisation campaign on the incidence and risk factors of acute hepatitis B in Italy. *J Hepatol* 2000;33:980-985.
  42. de Franchis R, Meucci G, Vecchi M, Tatarella M, Colombo M, Del Ninno E, Rumi NG, et al. The natural history of asymptomatic hepatitis B surface antigen carriers. *Ann Intern Med* 1993;118:191-194.
  43. McMahon BJ, Holck P, Bulkow L, Snowball MM. Serologic and clinical outcomes of 1536 Alaska Natives chronically infected with hepatitis B virus. *Ann Int Med* 2001;135:759-768.
  44. Colin JF, Cazals-Hatem D, Loriot MA, Martinot-Peignoux M, Pham BN, Auperin A, Degott C, et al. Influence of human immunodeficiency virus infection on chronic hepatitis B in homosexual men. *HEPATOLOGY* 1999;29:1306-1310.
  45. Dragosics B, Ferenci P, Hitchman E, Denk H. Long-term follow-up study of symptomatic HBsAg-positive voluntary blood donors in Austria: a clinical and histologic evaluation of 242 cases. *HEPATOLOGY* 1987;7:302-306.
  46. Davis GL, Hoofnagle JH, Waggoner JG. Spontaneous reactivation of chronic hepatitis B virus infection. *Gastroenterology* 1984;86:230-235.
  47. Liaw YF, Tai DI, Chu CM, Pao CC, Chen TJ. Acute exacerbation in chronic type B hepatitis: comparison between HBeAg and antibody-positive patients. *HEPATOLOGY* 1987;7:20-23.
  48. Fattovich G, Brollo L, Alberti A, Pontisso P, Giustina G, Realdi G. Long-term follow-up of anti-HBe-positive chronic active hepatitis B. *HEPATOLOGY* 1988;8:1651-1654.
  49. Chan HLY, Leung NWY, Hussain M, Wong ML, Lok ASF. Hepatitis B e antigen-negative chronic hepatitis B in Hong Kong. *HEPATOLOGY* 2000;31:763-768.
  50. Brunetto MR, Oliveri F, Rocca G, Criscuolo D, Chiaberge E, Capalbo M, David E, et al. Natural course and response to interferon of chronic hepatitis B accompanied by antibody to hepatitis B e antigen. *HEPATOLOGY* 1989;10:198-202.
  51. Lindh M, Andersson AS, Gusdal A. Genotypes, nt 1858 variants, and geographic origin of hepatitis B virus - large-scale analysis using a new genotyping method. *J Infect Dis* 1997;175:1285-1293.
  52. Laras A, Koskinas J, Avgidis K, Hadziyannis SJ. Incidence and clinical significance of hepatitis B virus precore gene translation initiation mutations in e antigen-negative patients. *J Viral Hepatitis* 1998;5:241-248.
  53. Naoumov NV, Schneider R, Grotzinger T, Jung MC, Miska S, Pape GR, Will H. Precore mutant hepatitis B virus infection and liver disease. *Gastroenterology* 1992;102:538-543.
  54. Rodriguez-Frias F, Buti M, Jardi R, Cotrina M, Viladomiu L, Esteban R, Guardia J. Hepatitis B virus infection: precore mutants and its relation to viral genotypes and core mutations. *HEPATOLOGY* 1995;22:1641-1647.
  55. Tu H, Xiong SD, Trepo C, Wen YM. Frequency of hepatitis B virus e-minus mutants varies among patients from different areas of China. *J Med Virol* 1997;51:85-89.
  56. Shindo M, Hamada K, Koya S, Sokawa Y, Okuno T. The clinical significance of core promoter and precore mutations during the natural course and interferon therapy in patients with chronic hepatitis B. *Am J Gastroenterol* 1999;94:237-245.
  57. Zarski JP, Marcellin P, Cohard M, Lutz JM, Bouche C, Rais A. Comparison of anti-HBe-positive and HBe-antigen-positive chronic hepatitis B in France. French Multicentre Group. *J Hepatol* 1994;20:636-640.
  58. Gray AH, Fang JW, Davis GL, Mizokami M, Wu PC, Williams R, Schuster SM, et al. Variations of hepatitis B virus core gene sequence in Western patients with chronic hepatitis B virus infection. *J Viral Hepatitis* 1997;4:371-378.
  59. Grandjacques C, Pradat P, Stuyver L, Chevallier M, Chevallier P, Pichoud C, Maisonnas M, et al. Rapid detection of genotypes and mutations in the pre-core promoter and the pre-core region of hepatitis B virus genome: correlation with viral persistence and disease severity. *J Hepatol* 2000;33:430-439.
  60. Hadziyannis S. Hepatitis B e antigen negative chronic hepatitis B: from clinical recognition to pathogenesis and treatment. *Viral Hepatitis Rev* 1995;1: 7-36.
  61. Di Marco V, Camma C, Vaccaro A, Giunta M, Martorana G, Fuschi P, Almasio P, et al. The long-term course of chronic hepatitis B. *HEPATOLOGY* 1999;30:257-264.
  62. Brunetto MR, Giarin MM, Oliveri F, Chiaberge E, Baldi M, Alfarano A, Serra A, et al. Wild-type and e antigen-minus hepatitis B viruses and course of chronic hepatitis. *Proc Natl Acad Sci U S A* 1991;88:4186-4190.
  63. Chu CM, Yeh CT, Chiu CT, Sheen IS, Liaw YF. Precore mutant of hepatitis B virus prevails in acute and chronic infections in an area in which hepatitis B is endemic. *J Clin Microbiol* 1996;34:1815-1818.
  64. Kramvis A, Kew MC, Bukofzer S. Hepatitis B virus precore mutants in serum and liver of Southern African blacks with hepatocellular carcinoma. *J Hepatol* 1998;28:132-141.
  65. Lok AS, Akarca U, Greene S. Mutations in the pre-core region of hepatitis B virus serve to enhance the stability of the secondary structure of the pre-genome encapsidation signal. *Proc Natl Acad Sci U S A* 1994;91:4077-4081.
  66. Carman WF, Jacyna MR, Hadziyannis S, Karayiannis P, McGarvey MJ, Makris A, Thomas HC. Mutation preventing formation of hepatitis B e antigen in patients with chronic hepatitis B infection. *Lancet* 1989;2: 588-591.
  67. Okamoto H, Tsuda F, Akahane Y, Sugai Y, Yoshida M, Moriyama K, Tanaka T, et al. Hepatitis B virus with mutations in the core promoter for an e antigen-negative phenotype in carriers with antibody to e antigen. *J Virol* 1994;68:8102-8110.
  68. Magnius LO, Nordner H. Subtypes, genotypes and molecular epidemiology of the hepatitis B virus as reflected by sequence variability of the S-gene. *Intervirology* 1995;38:24-34.
  69. Adachi J, Kaneko S, Matsushita E, Inagaki Y, Unoura M, Kobayashi K. Clearance of HBsAg in seven patients with chronic hepatitis. *HEPATOLOGY* 1992;16:1334-1337.

70. Liaw YF, Sheen IS, Chen TJ, Chu CM, Pao CC. Incidence, determinants and significance of delayed clearance of serum HBsAg in chronic hepatitis B virus infection: a prospective study. *HEPATOLOGY* 1991;13:627-631.
71. Gandhi MJ, Yang GG, McMahon B, Vyas G. Hepatitis B virions isolated with antibodies to the pre-S1 domain reveal occult viremia in surface antigen negative/antibody-positive carriers by polymerase chain reaction. *Transfusion* 2000;40:910-916.
72. Yu MW, Hsu FC, Sheen IS, Chu CM, Lin DY, Chen CJ, Liaw YF. Prospective study of hepatocellular carcinoma and liver cirrhosis in asymptomatic chronic hepatitis B virus carriers. *Am J Epidemiol* 1997;145:1039-1047.
73. Liaw YF, Tai DI, Chu CM, Chen TJ. The development of cirrhosis in patients with chronic type B hepatitis: a prospective study. *HEPATOLOGY* 1988;8:493-496.
74. Fattovich G, Brollo L, Giustina G, Noventa F, Pontisso P, Alberti A, Raldi G, et al. Natural history and prognostic factors for chronic hepatitis type B. *Gut* 1991;32:294-298.
75. Raldi G, Fattovich G, Hadziyannis S, Schalm SW, Almasio P, Sanchez-Tapias J, Christensen E, et al. Survival and prognostic factors in 366 patients with compensated cirrhosis type B: a multicenter study. The investigators of the European Concerted Action on Viral Hepatitis (EUROHEP). *J Hepatol* 1994;21:656-666.
76. De Jongh FE, Janssen HLA, De Man FA, Hop WCJ, Schalm SW, Van Blankenstein MV. Survival and prognostic indicators in hepatitis B surface antigen-positive cirrhosis of the liver. *Gastroenterology* 1992;103:1630-1635.
77. Fattovich G, Giustina G, Schalm SW, Hadziyannis S, Sanchez-Tapias J, Almasio P, Christensen E, et al. Occurrence of hepatocellular carcinoma and decompensation in western European patients with cirrhosis type B. The EUROHEP Study Group on Hepatitis B Virus and Cirrhosis. *HEPATOLOGY* 1995;21:77-82.
78. Niederau C, Heintges T, Lange S, Goldman G, Niederau CM, Mohr L, Haussinger D. Long-term follow-up of HBeAg-positive patients treated with interferon alfa for chronic hepatitis B. *N Engl J Med* 1996;334:1422-1427.
79. Fattovich G, Giustina G, Raldi G, Corrocher R, Schalm SW, and the European Concerted Action of Viral Hepatitis (EUROHEP). Long-term outcome of hepatitis B e antigen-positive patients with compensated cirrhosis treated with interferon alfa. *HEPATOLOGY* 1997;26:1338-1342.
80. Lin SM, Sheen IS, Chien RN, Chu CM, Liaw YF. Long-term beneficial effect of interferon therapy in patients with chronic hepatitis B virus infection. *HEPATOLOGY* 1999;29:971-975.
81. Lau DT, Everhart J, Kleiner DE, Park Y, Vergalla J, Schmid P, Hoofnagle JH. Long-term follow-up of patients with chronic hepatitis B treated with interferon alfa. *Gastroenterology* 1997;113:1660-1667.
82. Liaw YF, Lin DY, Chen TJ, Chu CM. Natural course after the development of cirrhosis in patients with chronic type B hepatitis: a prospective study. *Liver* 1989;9:235-241.
83. Chung HT, Lai CL, Lok AS. Pathogenic role of hepatitis B virus in hepatitis B surface antigen-negative decompensated cirrhosis. *HEPATOLOGY* 1995;22:25-29.
84. Huo TI, Wu JC, Lee PC, Chau GY, Lui WY, Tsai SH, Ting LT, et al. Sero-clearance of hepatitis B surface antigen in chronic carriers does not necessarily imply a good prognosis. *HEPATOLOGY* 1998;28:231-236.
85. Roudot-Thoraval F, Bastie A, Pawlotsky JM, Dhumeaux D, and the Study Group for the Prevalence and the Epidemiology of Hepatitis C Virus. Epidemiological factors affecting the severity of hepatitis C virus-related liver disease: a French survey of 6,664 patients. *HEPATOLOGY* 1997;26:485-490.
86. Housset C, Pol S, Carnot F, Dubois F, Nalpas B, Housset B, Berthelot P, et al. Interactions between human immunodeficiency virus-1, hepatitis delta virus and hepatitis B virus infections in 260 chronic carriers of hepatitis B virus. *HEPATOLOGY* 1992;15:578-592.
87. Hadziyannis SJ. Hepatitis D. *Clin Liver Dis* 1999;3:309-325.
88. Hadler SC, Alcalá de Monzon M, Rivero D, Perez M, Bracho A, Fields H. Epidemiology and long-term consequences of hepatitis Delta virus infection in the Yucpa Indians of Venezuela. *Am J Epidemiol* 1992;136:1507-1516.
89. Gaeta GB, Stroffolini T, Chiaramonte M, Ascione T, Sornaiuolo G, Lorello S, Sagnelli E, et al. Chronic hepatitis D: a vanishing disease? An Italian multinational study. *HEPATOLOGY* 2000;32:824-827.
90. Careda F, Rossi E, d'Arminio Monteforte A, Zampini L, Re T, Meroni B, Moroni M. Hepatitis B virus-associated coinfection and superinfection with delta agent: Indistinguishable disease with different outcome. *J Infect Dis* 1985;151:925-928.
91. Fattovich G, Boscaro S, Noventa F, Pornaro E, Stenico D, Alberti A, Ruol A, et al. Influence of hepatitis delta virus infection on progression to cirrhosis in chronic hepatitis type B. *J Infect Dis* 1987;155:931-935.
92. Fattovich G, Giustina G, Christensen E, Pantalena M, Zagni I, Raldi G, Schalm SW, and the European Concerted Action on Viral Hepatitis (Eurohep). Influence of hepatitis delta virus infection on morbidity and mortality in compensated cirrhosis type B. *Gut* 2000;46:420-426.
93. Centers for Disease Control and Prevention. Prevention of hepatitis A through active or passive immunization: recommendations of the Advisory Committee on Immunization Practices (ACIP). *MMWR Morb Mortal Wkly Rep* 1996;45:1-30.
94. Gerlich WH, Thomssen R. Quantitative assays for hepatitis B virus DNA: standardization and quality control. *Viral Hepatitis Reviews* 1995;1:53-57.
95. Hawkins A, Davidson F, Simmonds P. Comparison of plasma virus loads among individuals infected with hepatitis C virus (HCV) genotypes 1, 2, and 3 by Quantiplex HCV RNA assay versions 1 and 2, Roche Monitor assay, and an in-house limiting dilution method. *J Clin Microbiol* 1997;35:187-192.
96. Pawlotsky JM, Bastie A, Hezode C, Lonjon I, Darthuy F, Remire J, Dhumeaux D. Routine detection and quantification of hepatitis B virus DNA in clinical laboratories: performance of three commercial assays. *J Virol Methods* 2000;85:11-21.
97. Desmet VJ, Gerber M, Hoofnagle JH, Manns M, Scheuer PJ. Classification of chronic hepatitis: diagnosis, grading and staging. *HEPATOLOGY* 1994;19:1513-1520.
98. Knodell RG, Ishak KG, Black WC, Chen TS, Craig R, Kaplowitz N, Kiernan TW, et al. Formulation and application of a numerical scoring system for assessing histological activity in asymptomatic chronic active hepatitis. *HEPATOLOGY* 1981;1: 431-435.
99. Ishak K, Baptista A, Bianchi L, Callea F, De Groote J, Gudat F, Denk H, et al. Histological grading and staging of chronic hepatitis. *J Hepatol* 1995;22:696-699.
100. The French Metavir Cooperative Study Group. Intraobserver and interobserver variations in liver biopsy interpretation in patients with chronic hepatitis C. *HEPATOLOGY* 1994;20:15-20.
101. Weissberg JI, Andres LL, Smith CI, Weick S, Nichols JE, Garcia G, Robinson WS, et al. Survival in chronic hepatitis B. An analysis of 379 patients. *Ann Intern Med* 1984;101:613-616.
102. Villa E, Rubbiani L, Barchi T, Ferretti I, Grisendi A, De Palma M, Belletani S, et al. Susceptibility of chronic symptomless HBsAg carriers to ethanol-induced hepatic damage. *Lancet* 1982;2:1243-1245.
103. Kim YI, Heathcote J, Wanless IR. The hepatitis B carrier state—a follow-up study of 100 consecutive cases. *Clin Invest Med* 1987;10:383-387.
104. Chevillotte G, Durbec JP, Gerolami A, Berthezene P, Bidart JM, Camatte R. Interaction between hepatitis B virus and alcohol consumption in liver cirrhosis: an epidemiologic study. *Gastroenterology* 1983;85:141-145.
105. Imanishi T, Morikawa S, Ohmagari K, Kurihara S, Nishihata S, Kamiya T, Hayashida K, et al. The effect of habitual alcohol drinking on the development of type B chronic hepatitis, liver cirrhosis and hepatocellular carcinoma. *Jpn J Gastroenterol* 1988;85:692-698.
106. Chung HT, Lai CL, Wu PC, Lok ASF. Synergism of chronic alcoholism and hepatitis B infection in liver disease. *J Gastroenterol Hepatol* 1989;4:11-16.
107. Lok ASF, Lai CL, Wu PC. Prevalence of isolated antibody to hepatitis B core antigen in an area endemic for hepatitis B virus infection: Implications in hepatitis B vaccination programs. *HEPATOLOGY* 1988;8:766-770.
108. McMahon BJ, Parkinson AJ. Clinical significance and management when antibody to hepatitis B core antigen is the sole marker for HBV infection. *Viral Hepatitis Rev* 2000;6:229-236.
109. Wong VC, Ip HM, Reesink HW, Lelie PN. Prevention of the HBsAg carrier state in newborn infants of mothers who are chronic carriers of HBsAg and HBeAg by administration of hepatitis-B vaccine and hepatitis-B immunoglobulin. *Lancet* 1984;1:921-926.
110. Burk RD, Hwang LY, Ho GYF, Shafritz D, Beasley RP. Outcome of perinatal hepatitis B virus exposure is dependent on maternal virus load. *J Infect Dis* 1994;170:1418-1423.
111. Harpaz R, Von Seidlein L, Averhoff FM, Tormey MP, Sinha SD, Kotso-poulou K, Lambert SB, et al. Transmission of hepatitis B virus to multiple patients from a surgeon without evidence of inadequate infection control. *N Engl J Med* 1996;334:549-554.
112. Gerberding JL. The infected health care provider. *N Engl J Med* 1996;334:594-595.

113. CDC. Recommendations for preventing transmission of human immunodeficiency virus and hepatitis B virus to patients during exposure-prone invasive procedures. *MMWR* 1991;40:RR-8:1-7.
114. Heyward WL, Bender TR, Lanier AP, Francis DP, McMahon BJ, Maynard JE. Serologic markers of hepatitis B virus and alpha-fetoprotein levels preceding primary hepatocellular carcinoma in Alaskan Eskimos. *Lancet* 1982;2:889-891.
115. Johnson PJ, Williams R. Serum alpha-fetoprotein estimations and doubling time in hepatocellular carcinoma: Influence of therapy and possible value in early detection. *J Nat Cancer Inst* 1980;64:1329-1332.
116. Kaneko S, Unoura M, Kobayashi K. Early detection of hepatocellular carcinoma. In: Okuda K and Tabor E, eds. *Liver Cancer*. New York: Churchill Livingstone 1997;393-406.
117. Sheu JC, Sung JL, Chen DS, Yang PM, Lai MY, Lee CS, Hsu HC, et al. Growth rate of asymptomatic hepatocellular carcinoma and its clinical implications. *Gastroenterology* 1985;89:259-266.
118. Tang ZY, Yang BH, Zhou XD. Primary prevention of hepatocellular carcinoma. *J Gastroenterol Hepatol* 1995;10:683-690.
119. McMahon BJ, Bulkow L, Harpster A, Snowball M, Lanier A, Sacco F, Dunaway E, et al. Screening for hepatocellular carcinoma in Alaska Natives infected with chronic hepatitis B: a 16-year population-based study. *HEPATOLOGY* 2000;32:842-846.
120. Lee CS, Sheu JC, Wang M, Hsu HC. Long-term outcome after surgery for asymptomatic small hepatocellular carcinoma. *Br J Surg* 1996;83:330-333.
121. Mima S, Sekiya C, Kanagawa H, Kohyama H, Gotoh K, Mizuo H, Ijiri M, et al. Mass screening for hepatocellular carcinoma: experience in Hokkaido, Japan. *J Gastroenterol Hepatol* 1994;9:361-655.
122. Sherman M, Peltekian KM, Lee C. Screening for hepatocellular carcinoma in chronic carriers of hepatitis B virus: incidence and prevalence of hepatocellular carcinoma in a North American urban population. *HEPATOLOGY* 1995;22:432-437.
123. Sheu JC, Sung JL, Chen DS, Lai MY, Wang TH, Yu JY, Yang PM. Early detection of hepatocellular carcinoma by real-time ultrasonography. *Cancer* 1985;56:660-666.
124. Dusheiko GM, Hobbs KEF, Dick R, Burroughs AK. Treatment of small hepatocellular carcinomas. *Lancet* 1992;340:285-288.
125. Mor E, Kaspa RT, Sheiner P, Schwartz M. Treatment of hepatocellular carcinoma associated with cirrhosis in the era of liver transplantation. *Ann Intern Med* 1998;129:643-653.
126. Liu CL, Fan ST. Nonresectional therapies for hepatocellular carcinoma. *Am J Surg* 1997;173:358-365.
127. Murakami R, Yoshimatsu S, Yamashita Y, Matsukawa T, Takahashi M, Sagara K. Treatment of hepatocellular carcinoma: value of percutaneous microwave coagulation. *Am J Radiol* 1995;164:1159-1164.
128. Matsuzaki Y, Osuga T, Saito Y, Chuganji Y, Tanaka N, Shoda J, Tsuji H, et al. A new, effective, and safe therapeutic option using proton irradiation for hepatocellular carcinoma. *Gastroenterology* 1994;106:1032-1041.
129. Gazelle GS, Goldberg SN, Solbiati L, Livraghi T. Tumor ablation with radio-frequency energy. *Radiology* 2000;217:633-646.
130. Zoli M, Magalotti D, Bianchi G, Gueli C, Marchesini G, Pisi E. Efficacy of a surveillance program for early detection of hepatocellular carcinoma. *Cancer* 1996;78:977-985.
131. Oka H, Kurioka N, Kim K, Kanno T, Kuroki T, Mizoguchi Y, Kobayashi K. Prospective study of early detection of hepatocellular carcinoma in patients with cirrhosis. *HEPATOLOGY* 1990;12:680-687.
132. Cottone M, Turri M, Caltagirone M, Parisi P, Orlando A, Fiorentino G, Virdone R, et al. Screening for hepatocellular carcinoma in patients with Child's A cirrhosis: an 8 year prospective study by ultrasound and alpha-fetoprotein. *J Hepatol* 1994;21:1029-1034.
133. Colombo M, de Franchis R, Del Ninno, Sangiovanni A, De Fazio C, Tommasini M, Donato MF, et al. Hepatocellular carcinoma in Italian patients with cirrhosis. *N Engl J Med* 1991;325:675-680.
134. Tanaka S, Kitamura T, Nakanishi K, Okuda S, Yamazaki H, Hiyama T, Fujimoto I. Effectiveness of periodic checkup by ultrasonography for the early diagnosis of hepatocellular carcinoma. *Cancer* 1990;66:2210-2214.
135. Fujiyama S, Izuno K, Gohshi K, Shibata J, Sato T. Clinical usefulness of Des- $\gamma$ -carboxy prothombin in early diagnosis of hepatocellular carcinoma. *Dig Dis Science* 1991;36:1787-1792.
136. Tanabe Y, Ohnishi K, Nomura F, Iida S. Plasma abnormal prothrombin levels in patients with small hepatocellular carcinoma. *Am J Gastroenterol* 1988;83:1386-1389.
137. Tsai SL, Huang GT, Yang PM, Sheu JC, Sung JL, Chen DS. Plasma Des- $\gamma$ -carboxy prothombin in early stage of hepatocellular carcinoma. *HEPATOLOGY* 1990;11:481-487.
138. Xu K, Meng XY, Wu JW, Shen B, Shi YC, Wei Q. Diagnostic value of serum  $\gamma$ -glutamyl transferase isoenzyme for hepatocellular carcinoma: a 10-year study. *Am J Gastroenterology* 1992;87:991-995.
139. Takahashi H, Saibara T, Iwamura I, Tomita A, Maeda T, Onishi S, Yamamoto Y, et al. Serum  $\alpha$ -L-fucosidase activity and tumor size in hepatocellular carcinoma. *HEPATOLOGY* 1994;19:1414-1417.
140. Giardina MG, Matarazzo M, Morante R, Lucariello A, Varriale A, Guardasole V, De Marco G. Serum  $\alpha$ -L-fucosidase activity and early detection of hepatocellular carcinoma. *Cancer* 1998;83:2468-2474.
141. Mita Y, Aoyagi Y, Yanagi M, Suda T, Suzuki Y, Asakura H. The usefulness of determining Des- $\gamma$ -carboxy prothombin by sensitive enzyme immunoassay in the early diagnosis of patients with hepatocellular carcinoma. *Cancer* 1998;82:1643-1648.
142. Nomura F, Ishijima M, Kuwa K, Tanaka N, Nakai T, Ohnishi K. Serum Des-gamma-carboxy prothombin levels determined by a new generation of sensitive immunoassays in patients with small-sized hepatocellular carcinoma. *Am J Gastroenterol* 1999;94:650-654.
143. Yuen MF, Cheng CC, Laufer IJ, Lam SK, Ooi CGC, Lai CL. Early detection of hepatocellular carcinoma increases the chance of treatment: Hong Kong experience. *HEPATOLOGY* 2000;31:330-335.
144. Solmi L, Primerano AMM, Gandolfi L. Ultrasound follow-up of patients at risk for hepatocellular carcinoma: Results of a prospective study on 360 cases. *Am J Gastroenterol* 1996;91:1189-1194.
145. Kang JY, Lee TP, Yap I, Lun KC. Analysis of cost-effectiveness of different strategies for hepatocellular carcinoma screening in hepatitis B virus carriers. *J Gastroenterol Hepatol* 1992;7:463-468.
146. Mark DB, Hiatky MA, Califf RM, Naylor CD, Lee KL, Armstrong PW, Barbash G, et al. Cost effectiveness of thrombolytic therapy with tissue plasminogen activator as compared with streptokinase for acute myocardial infarction. *N Engl J Med* 1995;332:1418-1424.
147. Wong DK, Cheung AM, O'Rourke K, Naylor CD, Detsky AS, Heathcote J. Effect of alpha-interferon treatment in patients with hepatitis B e antigen-positive chronic hepatitis B. *Ann Intern Med* 1993;119:312-323.
148. Brook MG, Karayiannis P, Thomas HC. Which patients with chronic hepatitis B virus infection will respond to alpha-interferon therapy? *HEPATOLOGY* 1989;10:761-763.
149. Perrillo RP, Schiff ER, Davis GL, Bodenheimer HC, Jr., Lindsay K, Payne J, Dienstag JL, et al. A randomized, controlled trial of interferon alfa-2b alone and after prednisone withdrawal for the treatment of chronic hepatitis B. *N Engl J Med* 1990;323:295-301.
150. Lok AS, Wu PC, Lai CL, Lau JY, Leung EK, Wong LS, Ma OC, et al. A controlled trial of interferon with or without prednisone priming for chronic hepatitis B. *Gastroenterology* 1992;102:2091-2097.
151. Lai CL, Lok AS, Lin HJ, Wu PC, Yeoh EK, Yeung CY. Placebo-controlled trial of recombinant alpha 2-interferon in Chinese HBsAg-carrier children. *Lancet* 1987;2:877-880.
152. Lai CL, Lin HJ, Lau JN, Lok AS, Wu PC, Chung HT, Wong LK, et al. Effect of recombinant alpha 2 interferon with or without prednisone in Chinese HBsAg carrier children. *Q J Med* 1991;78:155-163.
153. Lok AS, Lai CL, Wu PC, Leung EK. Long-term follow-up in a randomized controlled trial of recombinant alpha 2-interferon in Chinese patients with chronic hepatitis B infection. *Lancet* 1988;2:298-302.
154. Gregorio GV, Jara P, Hierro L, Diaz C, de la Vega A, Vegnente A, Iorio R, et al. Lymphoblastoid interferon alfa with or without steroid pretreatment in children with chronic hepatitis B: a multicenter controlled trial. *HEPATOLOGY* 1996;23:700-707.
155. Sokal EM, Conjeevaram HS, Roberts EA, Alvarez F, Bern EM, Goyens P, Rosenthal P, et al. Interferon alfa therapy for chronic hepatitis B in children: a multinational randomized controlled trial. *Gastroenterology* 1998;114:988-995.
156. Jara P, Bortolotti F. Interferon-alpha treatment of chronic hepatitis B in childhood: a consensus advice based on experience in European children. *J Pediatr Gastroenterol Nutr* 1999;29:163-170.
157. Torre D, Tambini R. Interferon-alpha therapy for chronic hepatitis B in children: a meta-analysis. *Clin Infect Dis* 1996;23:131-137.
158. Lampertico P, Del Ninno E, Manzin A, Donato MF, Rumi MG, Lunghi G, Morabito A, et al. A randomized, controlled trial of a 24-month course of interferon alfa 2b in patients with chronic hepatitis B who had hepatitis B virus DNA without hepatitis B e antigen in serum. *HEPATOLOGY* 1997;26:1621-1625.
159. Fattovich G, Farci P, Rugge M, Brollo L, Mandas A, Pontisso P, Giustina G, et al. A randomized controlled trial of lymphoblastoid interferon-

- alpha in patients with chronic hepatitis B lacking HBeAg. *HEPATOLOGY* 1992;15:584-589.
160. Hadziyannis S, Bramou T, Makris A, Moussoulis G, Zignego L, Papaioannou C. Interferon alfa-2b treatment of HBeAg negative/serum HBV DNA positive chronic active hepatitis type B. *J Hepatol* 1990;11(Suppl 1):S133-S136.
161. Pastore G, Santantonio T, Milella M, Monno L, Mariano N, Moschetta R, Pollice L. Anti-HBe-positive chronic hepatitis B with HBV-DNA in the serum response to a 6-month course of lymphoblastoid interferon. *J Hepatol* 1992;14:221-225.
162. Oliveri F, Santantonio T, Bellati G, Colombatto P, Mels GC, Carriero L, Dastoli G, et al. Long term response to therapy of chronic anti-HBe-positive hepatitis B is poor independent of type and schedule of interferon. *Am J Gastroenterol* 1999;94:1366-1372.
163. Papatheodoridis GV, Manesis E, Hadziyannis SJ. Long-term follow up after initial response to interferon therapy in patients with HBeAg negative chronic hepatitis B (abstract). *HEPATOLOGY* 2000;32:378A.
164. Papatheodoridis GV, Manesis E, Hadziyannis SJ. The long-term outcome of interferon-alfa treated and untreated patients with HBeAg negative chronic hepatitis B. *J Hepatol* 2001;34:306-313.
165. Carreno V, Marcellin P, Hadziyannis S, Salmeron J, Diago M, Kitis GE, Vafiadis I, et al. Retreatment of chronic hepatitis B e antigen-positive patients with recombinant interferon alfa-2a. The European Concerted Action on Viral Hepatitis (EUROHEP). *HEPATOLOGY* 1999;30:277-282.
166. Perrillo R, Tamburro C, Regenstien F, Balart L, Bodenheimer H, Silva M, Schiff E, et al. Low-dose, titratable interferon alfa in decompensated liver disease caused by chronic infection with hepatitis B virus. *Gastroenterology* 1995;109:908-916.
167. Hoofnagle JH, Di Bisceglie AM, Waggoner JG, Park Y. Interferon alfa for patients with clinically apparent cirrhosis due to chronic hepatitis B. *Gastroenterology* 1993;104:1116-1121.
168. Saracco G, Mazzella G, Rosina F, Cancellieri C, Lattore V, Raise E, Rocca G, et al. A controlled trial of human lymphoblastoid interferon in chronic hepatitis B in Italy. *HEPATOLOGY* 1989;10:336-341.
169. Scully LJ, Shein R, Karayiannis P, McDonald JA, Thomas HC. Lymphoblastoid interferon therapy of chronic HBV infection. A comparison of 12 vs. 24 weeks of thrice weekly treatment. *J Hepatol* 1987;5:51-58.
170. Janssen HL, Gerken G, Carreno V, Marcellin P, Naoumov NV, Craxi A, Ring-Larsen H, et al. Interferon alfa for chronic hepatitis B infection: increased efficacy of prolonged treatment. The European Concerted Action on Viral Hepatitis (EUROHEP). *HEPATOLOGY* 1999;30:238-243.
171. Cohard M, Poynard T, Mathurin P, Zarski JP. Prednisone-interferon combination in the treatment of chronic hepatitis B: direct and indirect meta-analysis. *HEPATOLOGY* 1994;20:1390-1398.
172. Krogsgaard K, Marcellin P, Trepo C, Berthelot P, Sanchez-Tapias JM, Bassendine M, Tran A, et al. Prednisolone withdrawal therapy enhances the effect of human lymphoblastoid interferon in chronic hepatitis B. *INTERPRET Trial Group. J Hepatol* 1996;25:803-813.
173. Wong JB, Koff RS, Tine F, Pauker SG. Cost-effectiveness of interferon-alpha 2b treatment for hepatitis B e antigen-positive chronic hepatitis B. *Ann Intern Med* 1995;122:664-675.
174. Lok AS, Chung HT, Liu VW, Ma OC. Long-term follow-up of chronic hepatitis B patients treated with interferon alfa. *Gastroenterology* 1993;105:1833-1838.
175. Korenman J, Baker B, Waggoner J, Everhart JE, Di Bisceglie AM, Hoofnagle JH. Long-term remission of chronic hepatitis B after alpha-interferon therapy. *Ann Intern Med* 1991;114:629-634.
176. Krogsgaard K. The long-term effect of treatment with interferon-alpha 2a in chronic hepatitis B. The Long-Term Follow-up Investigator Group. The European Study Group on Viral Hepatitis (EUROHEP). Executive Team on Anti-Viral Treatment. *J Viral Hepat* 1998;5:389-397.
177. Carreno V, Castillo I, Molina J, Porres JC, Bartolome J. Long-term follow-up of hepatitis B chronic carriers who responded to interferon therapy. *J Hepatol* 1992;15:102-106.
178. Fong TL, Di Bisceglie AM, Gerber MA, Waggoner JG, Hoofnagle JH. Persistence of hepatitis B virus DNA in the liver after loss of HBsAg in chronic hepatitis B. *HEPATOLOGY* 1993;18:1313-1318.
179. Bortolotti F, Jara P, Barbera C, Gregorio GV, Vegnente A, Zancan L, Hierro L, et al. Long term effect of alpha interferon in children with chronic hepatitis B. *Gut* 2000;46:715-718.
180. Dienstag JL, Schiff ER, Wright TL, Perrillo RP, Hann HW, Goodman Z, Crowther L, et al. Lamivudine as Initial Treatment for Chronic Hepatitis B in the United States. *N Engl J Med* 1999;341:1256-1263.
181. Lai CL, Chien RN, Leung NW, Chang TT, Guan R, Tai DI, Ng KY, et al. A one-year trial of lamivudine for chronic hepatitis B. Asia Hepatitis Lamivudine Study Group. *N Engl J Med* 1998;339:61-68.
182. Schalm SW, Heathcote J, Cianciara J, Farrell G, Sherman M, Willems B, Dhillon A, et al. Lamivudine and alpha interferon combination treatment of patients with chronic hepatitis B infection: a randomised trial. *Gut* 2000;46:562-568.
183. Liaw YF, Leung NWY, Chang TT, Guan R, Tai DI, Ng KY, Chien RN, et al. Effects of extended lamivudine therapy in Asian patients with chronic hepatitis B. *Gastroenterology* 2000;119:172-180.
184. Leung NWY, Lai CL, Chang TT, Guan R, Lee CM, Ng KY, Wu PC, et al. Extended lamivudine treatment in patients with chronic hepatitis B enhances hepatitis B e antigen seroconversion rates: results after 3 years of therapy. *HEPATOLOGY* 2001;33:1527-1532.
185. Chang TT, Lai CL, Liaw YF, Guan R, Lim SG, Lee CM, Ng KY, et al. Incremental increases in HBeAg seroconversion and continued ALT normalization in Asian chronic HBV (CHB) patients treated with lamivudine for four years (abstract). *Antiviral Therapy* 2000;5(Suppl 1):44.
186. Chien RN, Liaw YF, Atkins M. Pretherapy alanine transaminase level as a determinant for hepatitis B e antigen seroconversion during lamivudine therapy in patients with chronic hepatitis B. Asia Hepatitis Lamivudine Trial Group. *HEPATOLOGY* 1999;30:770-774.
187. Perrillo RP, Schalm SW, Schiff ER, Brown NA, Woessner MA, Sullivan M. Predictors of HBsAg seroconversion in chronic hepatitis B patients treated with lamivudine (abstract). *HEPATOLOGY* 1999;30:317A.
- 187a. Sokal EM, Kelly D, Mizerski J, Badia I, Areias J, Schwarz K, Little N, Bell S, Greensmith MJ, Jonas M. An international double-blind placebo-controlled trial of lamivudine in 286 children with chronic hepatitis B (CHB). *J Hepatol* 2001;34(suppl 1):23A.
188. Tassopoulos NC, Volpes R, Pastore G, Heathcote J, Buti M, Goldin RD, Hawley S, et al. Efficacy of lamivudine in patients with hepatitis B e antigen-negative/hepatitis B virus DNA-positive (precore mutant) chronic hepatitis B. Lamivudine Precore Mutant Study Group. *HEPATOLOGY* 1999;29:889-896.
189. Santantonio T, Mazzola M, Iacovazzi T, Miglietta A, Guastadisegni A, Pastore G. Long-term follow-up of patients with anti-HBe/HBV DNA-positive chronic hepatitis B treated for 12 months with lamivudine. *J Hepatol* 2000;32:300-306.
190. Lok ASF, Hussain M, Cursano C, Margotti M, Gramenzi A, Grazi GL, Jovine E, et al. Evolution of hepatitis B virus polymerase gene mutations in hepatitis B e antigen-negative patients receiving lamivudine therapy. *HEPATOLOGY* 2000;32:1145-1153.
191. Hadziyannis SJ, Papatheodoridis GV, Dimou E, Laras A, Papaioannou C. Efficacy of long-term lamivudine monotherapy in patients with hepatitis B e antigen-negative chronic hepatitis B. *HEPATOLOGY* 2000;32:847-851.
192. Lau DT, Khokhar MF, Doo E, Ghany MG, Herion D, Park Y, Kleiner DE, et al. Long-term therapy of chronic hepatitis B with lamivudine. *HEPATOLOGY* 2000;32:828-834.
193. Rizzetto M, Volpes R, Smedile A. Response of pre-core mutant chronic hepatitis B infection to lamivudine. *J Med Virol* 2000;61:398-400.
194. Tassopoulos NC, Volpes R, Pastore G, Heathcote J, Buti M, Gray DF, Barber J, et al. Post lamivudine treatment follow-up of patients with HBeAg negative chronic hepatitis B (abstract). *J Hepatol* 1999;30(suppl 1):117.
195. Schiff E, Karayalcin S, Grimm I, Perrillo R, Dienstag J, Husa P, Schalm S, et al. A placebo controlled study of lamivudine and interferon alpha 2b in patients with chronic hepatitis B who previously failed interferon therapy (abstract). *HEPATOLOGY* 1998;28:388A.
196. Perrillo RP, Wright T, Rakela J, Levy G, Schiff E, Gish R, Martin P, et al. A multicenter United States-Canadian trial to assess lamivudine monotherapy before and after liver transplantation for chronic hepatitis B. *HEPATOLOGY* 2001;33:424-432.
197. Villeneuve JP, Condreay LD, Willems B, Pomier-Layrargues G, Fenyses D, Bilodeau M, Leduc R, et al. Lamivudine treatment for decompensated cirrhosis resulting from chronic hepatitis B. *HEPATOLOGY* 2000;31:207-210.
198. Yao FY, Bass NM. Lamivudine treatment in patients with severely decompensated cirrhosis due to replicating hepatitis B infection. *J Hepatol* 2000;33:301-307.
199. Fontana RJ, Perrillo R, Hann HW, Wright T, Rakela J, Bacon BR, Anshuetz G, et al. Determinants of survival in 133 patients with decompensated chronic hepatitis B treated with lamivudine (abstract). *HEPATOLOGY* 2000;32:221A.
200. Schiff E, Cianciara J, Karayalcin S, Kowdley K, Woesner M, McMullen S, Pearce M, et al. Durable HBeAg and HBsAg seroconversion after lamivudine for chronic hepatitis B (abstract). *J Hepatol* 2000;32(Suppl 2):99.



201. Song BC, Suh DJ, Lee HC, Chung YH, Lee YS. Hepatitis B e antigen seroconversion after lamivudine therapy is not durable in patients with chronic hepatitis B in Korea. *HEPATOLOGY* 2000;32:803-806.
202. Allen MI, Deslauriers M, Andrews CW, Tipples GA, Walters KA, Tyrrell DL, Brown N, et al. Identification and characterization of mutations in hepatitis B virus resistant to lamivudine. Lamivudine Clinical Investigation Group. *HEPATOLOGY* 1998;27:1670-1677.
203. Stuyver LJ, Locarnini SA, Lok A, Richman DD, Carman WF, Dienstag JL, Schinazi RF, et al. Nomenclature for antiviral-resistant human hepatitis B virus mutations in the polymerase region. *HEPATOLOGY* 2001;33:751-757.
204. Liaw YF, Chien RN, Yeh CT, Tsai SL, Chu CM. Acute exacerbation and hepatitis B virus clearance after emergence of YMDD motif mutation during lamivudine therapy. *HEPATOLOGY* 1999;30:567-572.
205. Bartholomew MM, Jansen RW, Jeffers LJ, Reddy KR, Johnson LC, Bunzendahl H, Condreay LD, et al. Hepatitis-B-virus resistance to lamivudine given for recurrent infection after orthotopic liver transplantation. *Lancet* 1997;349:20-22.
206. Tipples GA, Ma MM, Fischer KP, Bain VG, Kneteman NM, Tyrrell DL. Mutation in HBV RNA-dependent DNA polymerase confers resistance to lamivudine in vivo. *HEPATOLOGY* 1996;24:714-717.
207. Melegari M, Scaglioni PP, Wands JR. Hepatitis B virus mutants associated with 3TC and famciclovir administration are replication defective. *HEPATOLOGY* 1998;27:628-633.
208. Ono-Nita SK, Kato N, Shiratori Y, Masaki T, Lan KH, Carrilho FJ, Omata M. YMDD motif in hepatitis B virus DNA polymerase influences on replication and lamivudine resistance: A study by in vitro full-length viral DNA transfection. *HEPATOLOGY* 1999;29:939-945.
209. Dienstag JL, Perrillo RP, Schiff ER, Bartholomew M, Vicary C, Rubin M. A preliminary trial of lamivudine for chronic hepatitis B infection. *N Engl J Med* 1995;333:1657-1661.
210. Lai CL, Ching CK, Tung AK, Li E, Young J, Hill A, Wong BC, et al. Lamivudine is effective in suppressing hepatitis B virus DNA in Chinese hepatitis B surface antigen carriers: a placebo-controlled trial. *HEPATOLOGY* 1997;25:241-244.
211. Honkoop P, de Man RA, Niesters HG, Zondervan PE, Schalm SW. Acute exacerbation of chronic hepatitis B virus infection after withdrawal of lamivudine therapy. *HEPATOLOGY* 2000;32:635-639.
212. de Man RA, Marcellin P, Habal F, Desmond P, Wright T, Rose T, Jurewicz R, et al. A randomized, placebo-controlled study to evaluate the efficacy of 12-month famciclovir treatment in patients with chronic hepatitis B e antigen-positive hepatitis B. *HEPATOLOGY* 2000;32:413-417.
213. Aye TT, Bartholomeusz A, Shaw T, Bowden S, Breschkin A, McMillan J, Angus P, et al. Hepatitis B virus polymerase mutations during antiviral therapy in a patient following liver transplantation. *J Hepatol* 1997;26:1148-1153.
214. Heathcote EJ, Jeffers L, Wright T, Sherman M, Perrillo R, Sacks S, Carithers R, et al. Loss of serum HBV DNA and HBeAg and seroconversion following short-term (12 weeks) adefovir dipivoxil therapy in chronic hepatitis B: two placebo controlled phase II studies (abstract). *HEPATOLOGY* 1998;28:317A.
215. Xiong X, Flores C, Yang H, Toole JJ, Gibbs CS. Mutations in hepatitis B DNA polymerase associated with resistance to lamivudine do not confer resistance to adefovir in vitro. *HEPATOLOGY* 1998;28:1669-1673.
216. Perrillo R, Schiff E, Yoshida E, Statler A, Hirsch K, Wright T, Gutfreund K, et al. Adefovir dipivoxil for the treatment of lamivudine-resistant hepatitis B mutants. *HEPATOLOGY* 2000;32:129-134.
217. Gish RG, Leung NWY, Wright TL, Trinh H, Robertson AT, Harris JJ, Delehanty JT, et al. Anti-hepatitis B virus (HBV) activity and pharmacokinetics of FTC in a 2 month trial in HBV infected patients (abstract). *Gastroenterology* 1999;116:A1216.
218. de Man R, Wolters L, Nevens F, Chua D, Sherman M, Lai CL, Thomas N, et al. A study of oral entecavir given for 28 days in both treatment-naïve and pre-treated subjects with chronic hepatitis (abstract). *HEPATOLOGY* 2000;32:376A.
219. Ono-Nita SK, Kato N, Shiratori Y, Yoshida H, Kato J, Goto T, Schinazi RF, et al. Influence of B domain mutation (L528M) of the hepatitis B virus polymerase on replication ability and resistance to nucleoside analogues (abstract). *HEPATOLOGY* 2000;32:393A.
220. Andreone P, Cursaro C, Gramenzi A, Zavaglia C, Rezakovic I, Altomare E, Severini R, et al. A randomized controlled trial of thymosin-alpha1 versus interferon alfa treatment in patients with hepatitis B e antigen antibody- and hepatitis B virus DNA-positive chronic hepatitis B. *HEPATOLOGY* 1996;24:774-777.
221. Chien RN, Liaw YF, Chen TC, Yeh CT, Sheen IS. Efficacy of thymosin alpha1 in patients with chronic hepatitis B: a randomized, controlled trial. *HEPATOLOGY* 1998;27:1383-1387.
222. Mutchnick MG, Lindsay KL, Schiff ER, Cummings GD, Appelman HD, Peleman RR, Silva M, et al. Thymosin alpha1 treatment of chronic hepatitis B: results of a phase III multicentre, randomized, double-blind and placebo-controlled study. *J Viral Hepat* 1999;6:397-403.
223. Zavaglia C, Severini R, Tinelli C, Franzone JS, Airolidi A, Tempini S, Bettale G, et al. A randomized, controlled study of thymosin-alpha1 therapy in patients with anti-HBe, HBV-DNA-positive chronic hepatitis B. *Dig Dis Sci* 2000;45:690-696.
224. Lau GK, Tsiang M, Hou J, Yuen S, Carman WF, Zhang L, Gibbs CS, et al. Combination therapy with lamivudine and famciclovir for chronic hepatitis B-infected Chinese patients: a viral dynamics study. *HEPATOLOGY* 2000;32:394-399.
225. Rosina F, Pintus C, Meschievitz C, Rizzetto M. A randomized controlled trial of a 12-month course of recombinant human interferon-alpha in chronic delta (type D) hepatitis: a multicenter Italian study. *HEPATOLOGY* 1991;13:1052-1056.
226. Farci P, Mandas A, Coiana A, Lai ME, Desmet V, Van Eyken P, Gibo Y, et al. Treatment of chronic hepatitis D with interferon alfa-2a. *N Engl J Med* 1994;330:88-94.
227. Farci P, Chessa L, Peddis G, Strazzera R, Pascariello E, Scioscia R, Lai ME, et al. Influence of alpha interferon on the natural history of chronic hepatitis D: dissociation of histologic and virologic response (abstract). *HEPATOLOGY* 2000;32:222A.
228. Lau DT, Doo E, Park Y, Kleiner DE, Schmid P, Kuhns MC, Hoofnagle JH. Lamivudine for chronic delta hepatitis. *HEPATOLOGY* 1999;30:546-549.